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Primary Amino Acid Derivatives: Substitution of the 4′-*N*′-Benzylamide Site in (*R*)-*N*′-Benzyl 2-Amino-3-methylbutanamide, (*R*)-*N*′-Benzyl 2-Amino-3,3-dimethylbutanamide, and (*R*)-*N*′-Benzyl 2-Amino-3-methoxypropionamide Provides Potent Anticonvulsants with Pain-Attenuating Properties

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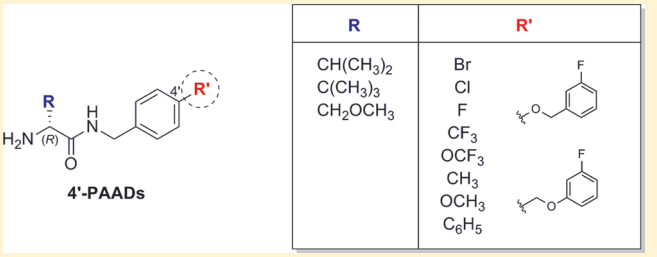
Supporting Information

ABSTRACT: Recently, we reported that select *N*′-benzyl 2-substituted 2-amino acetamides (primary amino acid derivatives (PAADs)) exhibited pronounced activities in established whole animal anticonvulsant (i.e., maximal electroshock seizure (MES)) and neuropathic pain (i.e., formalin) models. The anticonvulsant activities of C(2)-hydrocarbon *N*′-benzyl 2-amino acetamides (MES ED_{50} = 13−21 mg/kg) exceeded those of phenobarbital (ED_{50} = 22 mg/kg). Two additional studies defining the structure−activity relationship of PAADs are presented in this issue of the journal. In this study, we demonstrated that the anticonvulsant activities of (*R*)-*N*′-benzyl 2-amino-3-methylbutanamide and (*R*)-*N*′-benzyl 2-amino-3,3-dimethylbutanamide were sensitive to substituents at the 4′-*N*′-benzylamide site; electron-withdrawing groups retained activity, electron-donating groups led to a loss of activity, and incorporating either a 3-fluorobenzyloxy or 3-fluorophenoxymethyl group using a rationally designed multiple ligand approach improved activity. Additionally, we showed that substituents at the 4′-*N*′-benzylamide site of (*R*)-*N*′-benzyl 2-amino-3-methoxypropionamide also improved anticonvulsant activity, with the 3-fluorophenoxymethyl group providing the largest (∼4-fold) increase in activity (ED_{50} = 8.9 mg/kg), a value that surpassed phenytoin (ED_{50} = 9.5 mg/kg). Collectively, the pharmacological findings provided new information that C(2)-hydrocarbon PAADs represent a novel class of anticonvulsants.



INTRODUCTION

Epilepsy and neuropathic pain are chronic neurological disorders that arise from dysregulations in neuronal function, including neuronal hyperexcitability and hypersynchronous neuronal firing.[1,2] Pharmacological management remains the primary treatment option,[3] and the similar pathophysiology of these disorders result in the clinical application of antiepileptic drugs (AEDs) to treat neuropathic pain.[4] Although newer generation AEDs have made improvements in patient care, pharmacoresistance (failure to respond to two first-line AEDs)[5] and adverse side effects (e.g., drowsiness, dizziness, nausea)[6] limit their therapeutic usefulness. The development of new compounds with a novel mechanism of action may circumvent the shortcomings of current AEDs.

We recently reported[7] that select primary amino acid derivatives (PAADs, **1**) displayed potent activities in established animal models of seizures (maximal electroshock seizure (MES)[8])[9] and neuropathic pain (formalin).[10] Our investigation of PAAD activity was prompted by the excellent pharmacological properties observed for their *N*-acetylated counterparts, the functionalized amino acids (FAAs, **2**),[11−22] from which lacosamide (LCM, (*R*)-**3**)[12] emerged as a first-in-class AED that is currently marketed in the US and Europe for the adjuvant treatment of partial-onset seizures in adults.[23] Several patch-clamp electrophysiology studies have shown that (*R*)-**3** enhances slow

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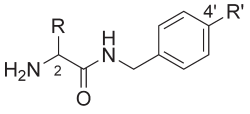
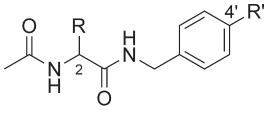
inactivation of voltage-gated sodium channels without affecting fast inactivation, a pharmacological process that helps to regulate the firing frequency of hyperexcitable neurons.[24−26] We found that the structure−activity relationship (SAR) for **1** and **2** differed, suggesting that their underlying mechanism(s) of action might differ as well. The C(2)-hydrocarbon PAADs (**4**) were the most active compounds in this study, and they surpassed the activities of the corresponding C(3)-*O*-alkoxy PAADs (**5**). This trend was unexpected because anticonvulsant activity improved in the FAA series when a substituted heteroatom was incorporated one atom removed from the chiral C(2) center.[11,12,17−19]



1 R' = H **(PAADs)**



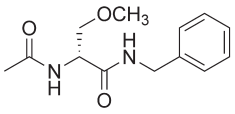
2 R' = H **(FAAs)**

4 R = hydrocarbon; R' = H

5 R = CH_2OR''; R' = H

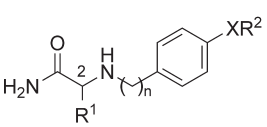
6 R = hydrocarbon; R' ≠ H

7 R = CH_2OCH_3; R' ≠ H

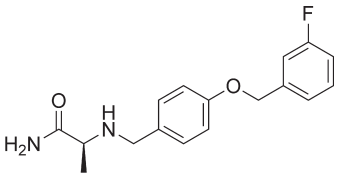


(*R*)-**3** (lacosamide)

In this study, we ask whether 4′-substitution of the *N*′-benzylamide moiety in C(2)-hydrocarbon PAADs **4** and C(3)-*O*-alkoxy PAADs **5**, to give PAADs **6** and **7**, would lead to improved pharmacological activity. We assessed anticonvulsant activity using the MES and 6 Hz psychomotor assays and, when possible, evaluated pain attenuation in the late (chronic) phase of the formalin neuropathic pain model. The 4′-substituents (R′) tested included small electron-donating (ED) or electron-withdrawing (EW) groups, as well as more complex groups using a rationally designed multiple ligand (DML) approach.[27] The combination of two ligands within a single agent to produce dual or chimeric agents has been used in a number of drug design programs[28−33] and has been shown to affectively modulate multiple targets at the same time (polypharmacology). In our study, we overlapped the commonalities of PAADs (**1**) and α-aminoamides (AAAs, **8**), a class of amino acid-based anticonvulsants that exhibit excellent activity in various animal seizure models,[34,35] to create chimeric PAADs. The archetypal AAA, safinamide ((*S*)-**9**), was initially discovered because of its anticonvulsant activity, and it has been advanced for the treatment of Parkinson's disease.[34,36−38]

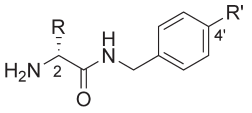


8
AAAs



(*S*)-**9**
(safinamide)

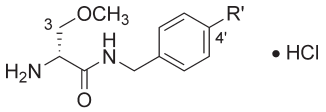
We demonstrated that PAAD anticonvulsant activity (MES test) for the C(2)-hydrocarbon PAADs **6** was markedly affected by the electronic properties of the 4′-*N*′-benzylamide substituent,



(*R*)-**10** R = $CH(CH_3)_2$ R' = H
(*R*)-**13** R = $CH(CH_3)_2$ R' = F
(*R*)-**14** R = $CH(CH_3)_2$ R' = Cl
(*R*)-**15** R = $CH(CH_3)_2$ R' = CH_3
(*R*)-**16** R = $CH(CH_3)_2$ R' = CF_3
(*R*)-**17** R = $CH(CH_3)_2$ R' = OCH_3
(*R*)-**18** R = $CH(CH_3)_2$ R' = OCF_3
(*R*)-**19** R = $CH(CH_3)_2$ R' = C_6H_5

(*R*)-**11** R = $C(CH_3)_3$ R' = H
(*R*)-**20** R = $C(CH_3)_3$ R' = Cl
(*R*)-**21** R = $C(CH_3)_3$ R' = CF_3
(*R*)-**22** R = $C(CH_3)_3$ R' = OCF_3
(*R*)-**23** R = $C(CH_3)_3$ R' = C_6H_5

Figure 1. 4′-*N*′-Benzylamide-substituted C(2)-hydrocarbon PAADs (series 1).



(*R*)-**12** R' = H
(*R*)-**24** R' = Br
(*R*)-**25** R' = CF_3
(*R*)-**26** R' = OCF_3
(*R*)-**27** R' = C_6H_5

Figure 2. 4′-*N*′-Benzylamide-substituted C(3)-*O*-methoxy PAADs (series 2).

in which EW groups largely retained excellent anticonvulsant activity and ED groups were inactive. We noted that while none of the 4′-EW substituents for **6** increased the anticonvulsant activity compared with the parent, unsubstituted PAADs, activity was enhanced through the DML strategy for PAADs **6** and **7**. The activity of the chimeric PAADs rivaled that found for most clinical AEDs. Moreover, where testing permitted, we showed that 4′-*N*′-benzylamide-substituted PAADs also exhibited potent activity in the formalin model of neuropathic pain.

RESULTS AND DISCUSSION

Choice of Compounds. The 4′-unsubstituted *N*′-benzylamide PAADs (*R*)-**10**−**12** served as the parent compounds in this study (Figures 1 and 2). We systematically modified the 4′-*N*′-benzylamide position according to four categories: 4′-*N*′-benzylamide-substituted C(2)-hydrocarbon PAADs ((*R*)-**13**−**23**) (series 1, Figure 1); 4′-*N*′-benzylamide-substituted C(3)-*O*-methoxy PAADs ((*R*)-**24**−**27**) (series 2, Figure 2); 4′-chimeric C(2)-hydrocarbon PAADs ((*R*)-**28**−**32**) (series 3, Figure 3); 4′-chimeric C(3)-*O*-methoxy PAADs ((*R*)-**33** and (*R*)-**34**) (series 4, Figure 3). Our initial study documented that the C(2) stereochemical preference corresponded to the D-amino acid ((*R*)-configuration).[7] Accordingly, we prepared the 4′-*N*′-benzylamide-substituted PAADs in the (*R*)-configuration.

Within the 4′-*N*′-benzylamide-substituted C(2)-hydrocarbon PAADs (series 1), we focused on C(2)-isopropyl and C(2)-*tert*-butyl derivatives because the parent, unsubstituted PAADs (*R*)-**10** (ED_{50} = 15 mg/kg) and (*R*)-**11** (ED_{50} = 14 mg/kg) exhibited excellent activities in the MES test (mice, ip).[7] For C(2)-isopropyl PAADs, the 4′-*N*′-benzylamide position (R′) of (*R*)-**10** was systematically modified with a fluoro, chloro, methyl, trifluoromethyl, methoxy, trifluoromethoxy, and phenyl group

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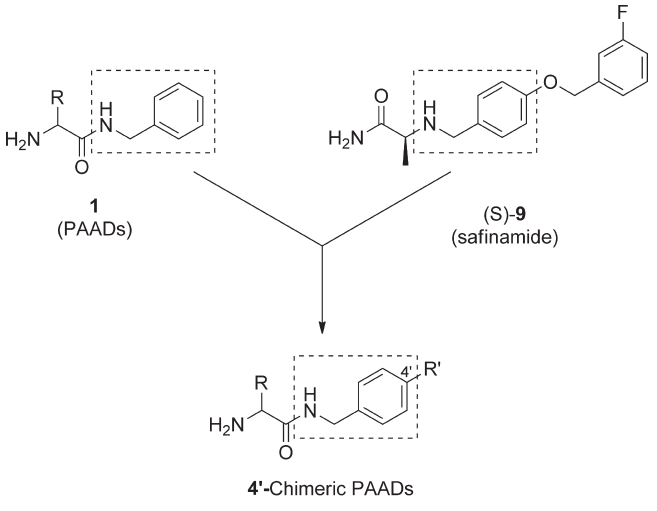


(*R*)-**28** R = CH_3 R' = $OCH_2C_6H_4$(*m*F)
(*R*)-**29** R = $CH(CH_3)_2$ R' = $OCH_2C_6H_4$(*m*F)
(*R*)-, (*S*)-**30** R = $CH(CH_3)_2$ R' = $CH_2OC_6H_4$(*m*F)
(*R*)-**31** R = $C(CH_3)_3$ R' = $OCH_2C_6H_4$(*m*F)
(*R*)-**32** R = $C(CH_3)_3$ R' = $CH_2OC_6H_4$(*m*F)

(*R*)-**33** R = CH_2OCH_3 R' = $OCH_2C_6H_4$(*m*F)
(*R*)-, (*S*)-**34** R = CH_2OCH_3 R' = $CH_2OC_6H_4$(*m*F)

Figure 3. 4′-Chimeric PAADs (series 3 and 4).

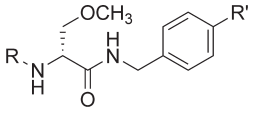
((*R*)-**13**−**19**) (Figure 1). Similarly, in the C(2)-*tert*-butyl PAAD set, the 4′-*N*′-benzylamide position of (*R*)-**11** was modified with a chloro, trifluoromethyl, trifluoromethoxy, and phenyl group ((*R*)-**20**−**23**). This series permitted us to assess the importance of size, electronics, and hydrophobic interactions of the 4′-substituents on anticonvulsant activity.

A smaller series of 4′-*N*′-benzylamide-substituted C(3)-*O*-methoxy PAADs (series 2) was prepared because we had observed a lower level of anticonvulsant activity in the MES test (mice, ip) for (*R*)-**12** (ED_{50} = 34 mg/kg) than for either (*R*)-**10** (ED_{50} = 15 mg/kg) or (*R*)-**11** (ED_{50} = 14 mg/kg).[7] Accordingly, we systematically modified the 4′-*N*′-benzylamide position (R′) of (*R*)-**12** with a bromo, trifluoromethyl, trifluoromethoxy, and phenyl group ((*R*)-**24**−**27**) (Figure 2). The compounds were purified and tested as their hydrochloride (HCl) salts.

Our pharmacological data for (*R*)-**13**−**27** indictated that small substituents at the 4′-*N*′-benzylamide position of C(2)-hydrocarbon and C(3)-*O*-methoxy PAADs affected the anticonvulsant activity. In the third and fourth series of 4′-*N*′-benzylamide-substituted PAADs, we evaluated the effect of a substantially larger substituent utilizing a DML approach in an effort to improve anticonvulsant activity and neuropathic pain attenuation. We overlaid the *N*-benzylamino unit in PAADs (**1**) and AAAs (**8**) (see structural unit in box in **1** and (*S*)-**9** in Figure 3) to create chimeric PAADs (*R*)-**28**−**34**. Each chimeric PAAD was defined by the R and R′ moieties, where R consisted of the PAAD functionality and R′ consisted of the AAA functionality. In series 3, R was a hydrocarbon (CH_3, $CH(CH_3)_2$, or $C(CH_3)_3$), and in series 4, R was CH_2OCH_3. In both series, R′ was either a 3-fluorobenzyloxy group or a 3-fluorophenoxymethyl group. All chimeric PAADs were synthesized in the (*R*)-configuration. The excellent activities of (*R*)-**30** and (*R*)-**34** prompted the synthesis of the (*S*)-stereoisomers to determine if the anticonvulsant activity of chimeric PAADs resided predominantly in the (*R*)-stereoisomer.

Chemistry. The 4′-*N*′-benzylamide-substituted C(2)-hydrocarbon PAADs (*R*)-**13**−**23** were synthesized by coupling either (*R*)-**35**[7] or (*R*)-**36**[7] with commercially available 4′-substituted benzylamines (**37**−**43**) using the standard mixed anhydride coupling (MAC) procedure,[39] followed by trifluoroacetic acid (TFA) deprotection (Scheme 1).

The 4′-*N*′-benzylamide-substituted C(3)-*O*-methoxy PAADs (*R*)-**24**[22] and (*R*)-**27**[22] were prepared by TFA deprotection of the *t*Boc-protected amides (*R*)-**55**[22] and (*R*)-**58**,[22] while catalytic removal (10% Pd−C, H_2) of the 2-*N*-(benzyloxycarbonyl) group in Cbz-protected amides (*R*)-**56**[22] and (*R*)-**57**[22] gave PAADs (*R*)-**25** and (*R*)-**26**.



(*R*)-**55** R = *t*Boc R' = Br

(*R*)-**56** R = Cbz R' = CF_3

(*R*)-**57** R = Cbz R' = OCF_3

(*R*)-**58** R = *t*Boc R' = C_6H_5

The 4′-chimeric C(2)-hydrocarbon PAADs (*R*)-**28**, (*R*)-**29**, (*R*)-**30**, (*S*)-**30**, (*R*)-**31**,[40] and (*R*)-**32** were synthesized from either (*R*)-**59**, (*R*)-**35**, (*S*)-**35**, or (*R*)-**36** following standard MAC procedures and using the appropriate 4′-modified benzylamines **61**[40] or **62**[40] (Scheme 2). PAADs (*R*)-**33**, (*R*)-**34**, and (*S*)-**34** were synthesized in the same manner beginning with (*R*)- and (*S*)-**60**, except alkylation of (*R*)-**68**,[40] (*R*)-**69**,[40] and (*S*)-**69** using methyl iodide and Ag_2O gave (*R*)-**70**,[40] (*R*)-**71**,[40] and (*S*)-**71**, respectively, before TFA deprotection to the corresponding PAADs.

In the Experimental Section, we detail the final step (synthetic procedure and characterization) for all compounds evaluated in the animal models. In the Supporting Information, we provide our experimental procedures for all prepared compounds and their physical and spectroscopic properties.

Pharmacological Activity. PAADs **13**−**34** were evaluated for anticonvulsant activity using the MES test at either UCB Pharma, following the procedures described by Klitgaard,[41] or at the National Institute of Neurological Disorders and Stroke Anticonvulsant Screening Program (NINDS ASP), following the procedures described by Stables and Kupferberg,[8] or both. Anticonvulsant activity using the 6 Hz test was performed either at UCB Pharma, following the procedures described by Kaminski and co-workers (44 mA),[42] or at the NINDS ASP, following the procedures described by Stables and Kupferberg (32 mA),[8] or both. Several PAADs evaluated at UCB Pharma and the NINDS ASP were also tested in the formalin model of neuropathic pain.[10,43] The formalin model of neuropathic pain is an effective tool to prescreen compounds for analgesic activity. Using drugs with pain-attenuating effects in humans, Vissers and co-workers demonstrated a good correlation between the findings in the second phase of the formalin test and the results for cold allodynia in the chronic constriction injury (CCI) model for both rats (r = 0.72) and gerbils (r = 0.68).[44] Therefore, the formalin model is

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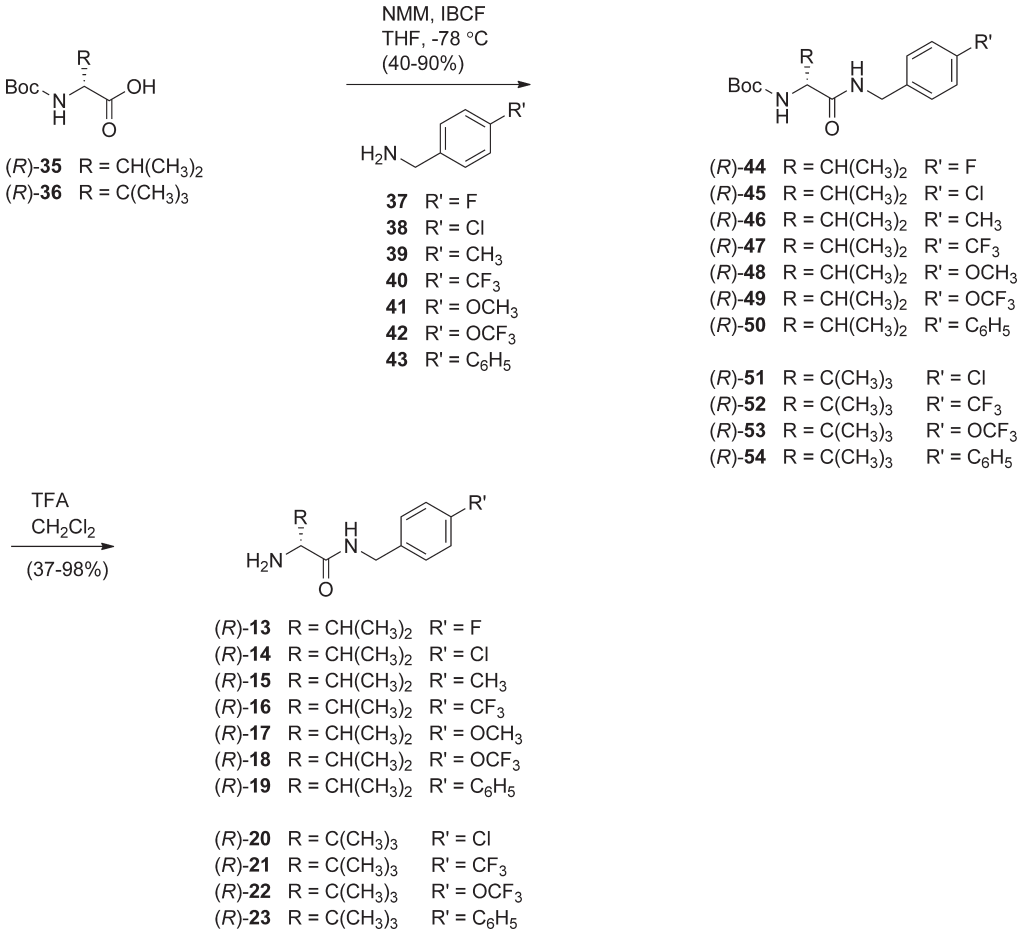
Scheme 1. Synthesis of 4′-*N*′-Benzylamide-Substituted Hydrocarbon PAADs (*R*)-13−23



comparable with other well-characterized models of neuropathic pain (e.g., CCI) and is advantageous due to the ease of administration and standardization. All compounds were administered intraperitoneally (ip) to mice at UCB Pharma and the NINDS ASP or orally (po) to rats at the NINDS ASP. The pharmacological data from the MES, 6 Hz, and formalin tests are summarized in Tables 1−4. The tables list the results obtained from either qualitative (dose range) or quantitative (ED_{50}) testing in mice (ip) and rats (po). We also included qualitative (dose range) or median neurological impairing dose (TD_{50}) values using the rotorod tes in mice (ip) and the positional sense test or gait and stance test in rats (po). ED_{50} and TD_{50} values were calculated from dose−response curves containing 4−6 dose points (n = 8−10 per dose). The protective indices (PI = TD_{50}/ED_{50}) were provided, when applicable. PAADs tested at the NINDS ASP were evaluated in the subcutaneous metrazol (scMet) seizure model[8] and displayed little to no protection.[45]

The C(2)-hydrocarbon PAADs (*R*)-**10** (ED_{50} = 15 mg/kg)[7] and (*R*)-**11** (ED_{50} = 14 mg/kg)[7] displayed excellent activity in the MES test that surpassed the activity of the traditional antiepileptic phenobarbital (ED_{50} = 22 mg/kg).[46] Additionally, (*R*)-**10** displayed significant activity (ED_{50} = 15 mg/kg) in the late (chronic) phase of the formalin model of neuropathic pain. Therefore, we chose to expand the SAR of (*R*)-**10** and (*R*)-**11** to include functionalization of the *N*′-benzylamide moiety in an attempt to optimize anticonvulsant activity and assess pain attenuation, when possible. We gauged the importance of electronic effects, hydrophobic interactions, and size of the 4′-substituent in C(2)-hydrocarbon PAADs (*R*)-**13**−**23** (series 1) on anticonvulsant activity in mice (Table 1). The MES activity for the 4′-chloro-substituted C(2)-isopropyl ((*R*)-**14**) and C(2)-*tert*-butyl ((*R*)-**20**) PAADs were similar (ED_{50} (mg/kg): (*R*)-**14**, 22; (*R*)-**20**, 25) but both were slightly lower than their respective parent compounds (*R*)-**10** and (*R*)-**11** (ED_{50} (mg/kg): (*R*)-**10**, 15; (*R*)-**11**, 14). The 4′-fluoro derivative (*R*)-**13** was evaluated in the isopropyl series and displayed slightly lower activity than the 4′-chloro derivative (*R*)-**14** (ED_{50} (mg/kg): (*R*)-**13**, 32; (*R*)-**14**, 22). Therefore, the EW effects of halogens (F and Cl) slightly decreased anticonvulsant activity compared with the unsubstituted parent compounds but, nonetheless, provided substantial anticonvulsant activity. Halogenated PAADs (*R*)-**13**, (*R*)-**14**, and (*R*)-**20** displayed significant activity in the formalin test (ED_{50} (mg/kg): (*R*)-**13**, 32; (*R*)-**14**, 22; (*R*)-**20**, 25) (Table 1). In rats (Table 1), the 4′-fluoro-substituted isopropyl PAAD (*R*)-**13** resulted in a 2-fold decrease in seizure protection from the parent PAAD (*R*)-**10** (ED_{50} (mg/kg): (*R*)-**10**, 11; (*R*)-**13**, 21). Next, we compared the 4′-methyl-substitued C(2)-isopropyl PAAD (*R*)-**15** and the 4′-trifluoromethyl analogue (*R*)-**16** in mice. The ED 4′-methyl moiety resulted in the complete loss of anticonvulsant activity in the MES test (ED_{50} = >300 mg/kg), while the EW 4′-trifluoromethyl moiety displayed excellent activity (ED_{50} = 14 mg/kg) (Table 1). Similar results were obtained when we evaluated the 4′-methoxy ((*R*)-**17**) and 4′-trifluoromethoxy ((*R*)-**18**)

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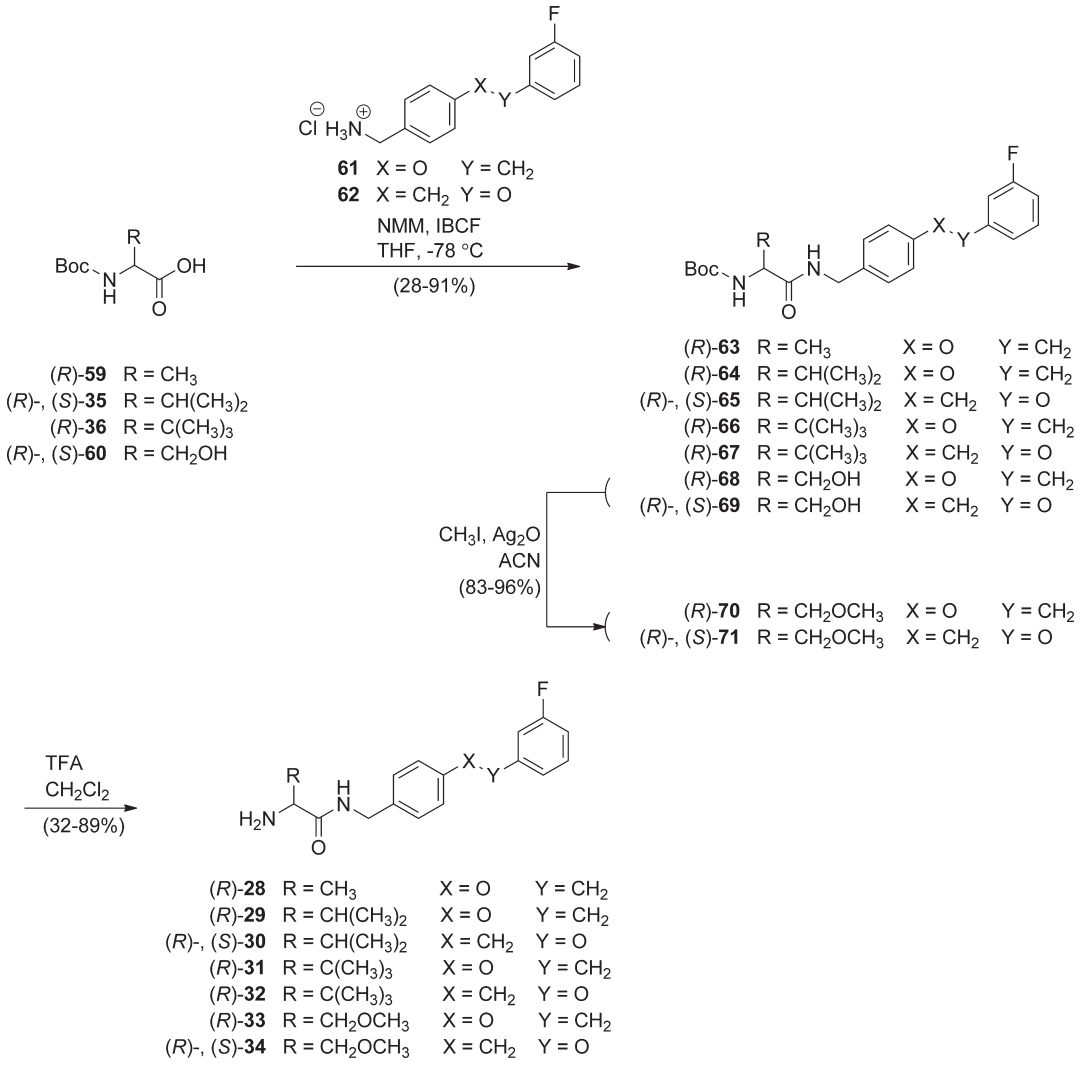
Scheme 2. Synthesis of 4′-Chimeric PAADs (*R*)-28−34, (*S*)-30, and (*S*)-34



derivatives in the C(2)-isopropyl series (ED_{50} (mg/kg): (*R*)-**17**, >300; (*R*)-**18**, 16). The 4′-trifluoromethyl ((*R*)-**21**) and 4′-trifluoromethoxy ((*R*)-**22**) compounds also displayed significant anticonvulsant activities in the C(2)-*tert*-butyl series (ED_{50} (mg/kg): (*R*)-**21**, 24; (*R*)-**22**, 28), although to a lesser degree than found in the isopropyl series, and were less active than the parent compound (*R*)-**11** (ED_{50} = 14 mg/kg). (*R*)-**16**, (*R*)-**18**, (*R*)-**21**, and (*R*)-**22** also showed substantial MES activity in the rat (ED_{50} (mg/kg): (*R*)-**16**, 13; (*R*)-**18**, 18; (*R*)-**21**, <30; (*R*)-**22**, 23) (Table 1). Additionally, the 4′-trifluoromethoxy PAAD (*R*)-**18** showed excellent activity in the formalin test (ED_{50} = 16 mg/kg) (Table 1). Finally, the 4′-phenyl-substituted derivatives (*R*)-**19** and (*R*)-**23** displayed moderate anticonvulsant activity in mice (ED_{50} = >30, <100 mg/kg).

Our data indicated that the electronic properties of 4′-*N*′-benzylamide substituents impacted pharmacological activity to a greater extent than the hydrophobic properties. EW 4′-substituents (defined as a positive Hammett σ_p value)[47] (Supporting Information Table S1) maintained seizure protection while ED groups (defined as a negative Hammett σ_p value) at this site dramatically decreased activity. Many, but not all, of the EW moieties contained fluorine substituents, and the benefit of incorporating fluorine substituents in CNS agents has been previously noted.[48] The greatest activity was observed in C(2)-isopropyl PAADs containing a CF_3 ((*R*)-**16**) or OCF_3 ((*R*)-**18**) group at the 4′-*N*-benzyl position. When the corresponding nonfluorinated moieties CH_3 ((*R*)-**15**) and OCH_3 ((*R*)-**17**) were incorporated at this position, the compounds were inactive, suggesting that electronic factors and, to a lesser extent, hydrophobic interactions (defined by their π-value)[47] (Supporting Information Table S1) may also have contributed to their anticonvulsant activity. However, there was not a direct correlation between hydrophobicity and pharmacological activity.

The correlation between the pharmacological activities of C(2)-hydrocarbon PAADs and the electronic properties of the 4′-substitutent was unexpected. In a related study, we showed that for 4′-substituted derivatives of the FAA (*R*)-**3**, anticonvulsant activity did not correlate with the electronic properties of the 4′-group, and that both ED and EW substituents displayed excellent protection in the MES model.[22] The sensitivity of C(2)-hydrocarbon PAADs to the electronic properties of the 4′-*N*-benzylamide substituent differentiates the SAR of our series from that reported for the FAA (*R*)-**3**.

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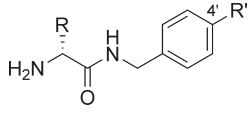
Table 1. Pharmacological Activities of 4′-*N*′-Benzylamide-Substituted C(2)-Hydrocarbon PAADs (Series 1) in Mice and Rats (mg/kg)



			mice (ip)[a]					rat (po)[b]		
compd	R	R′	MES,[c] ED_{50}	6 Hz,[d] ED_{50}	Formalin, ED_{50}	Tox,[e] TD_{50}	PI,[f] MES	MES,[c] ED_{50}	Tox,[g] TD_{50}	PI[f]
(*R*)-**10**[h]	$CH(CH_3)_2$	H	15 [0.25] (13−18)	<100 [0.25−1.0]	15 [0.25]	70 [0.25] (63−80)	4.8	11 [0.25] (9.1−13)	>500	>45
(*R*)-**13**	$CH(CH_3)_2$	F	32 [0.25] (28−39)	63 [0.25] (45−89)	32 [0.25]	97 [0.25] (88−105)	3.0	21 [0.5] (13−31)	>500	>24
(*R*)-**14**	$CH(CH_3)_2$	Cl	22 [0.25] (20−25)	∼50 [0.25]	22 [0.25]	74 [0.25] (72−78)	3.4	ND[i]	ND[i]	
(*R*)-**15**	$CH(CH_3)_2$	CH_3	>300 [0.5]	ND[i]	ND[i]	>300 [0.5]		∼50 [1.0−2.0]	>50 [0.25−0.5]	
(*R*)-**16**	$CH(CH_3)_2$	CF_3	14 [0.5] (12−16)	30 [0.5] (20−42)	ND[i]	57 [0.25] (54−64)	4.1	13 [1.0] (9−18)	>500	>38
(*R*)-**17**	$CH(CH_3)_2$	OCH_3	>300 [0.5]	ND[i]	ND[i]	>30, <100 [0.5]		>30 [0.25−4.0]	>30 [0.25−4.0]	
(*R*)-**18**	$CH(CH_3)_2$	OCF_3	16 [0.25] (14−20)	28 [0.5] (18−40)	16 [0.25]	84 [0.25] (67−109)	5.3	18 [1.0] (12−28)	>500	>27
(*R*)-**19**	$CH(CH_3)_2$	C_6H_5	>30, <100 [0.5]	ND[i]	ND[i]	>300 [0.5]		∼30 [2.0]	>30 [0.25−4.0]	
(*R*)-**11**[h]	$C(CH_3)_3$	H	14 [0.25] (11−17)	∼30 [0.25−0.5]	ND[i]	66 [0.25] (58−73)	4.7	11 [0.25] (8.7−15)	>500	>45
(*R*)-**20**	$C(CH_3)_3$	Cl	25 [0.25] (21−29)	<100 [0.25−2.0]	25 [0.25]	84 [0.5] (75−100)	3.4	∼30 [0.25−0.5]	>30 [0.25−4.0]	
(*R*)-**21**	$C(CH_3)_3$	CF_3	24 [0.25] (21−28)	ND[i]	ND[i]	133 [0.25] (93−197)	5.5	<30 [0.5−4.0]	>30 [0.25−4.0]	
(*R*)-**22**	$C(CH_3)_3$	OCF_3	28 [1.0] (22−34)	ND[i]	ND[i]	73 [0.25] (60−86)	2.6	23 [2.0] (17−33)	>30 [0.25−4.0]	
(*R*)-**23**	$C(CH_3)_3$	C_6H_5	>30, <100 [0.5]	ND[i]	ND[i]	>30, <100 [0.5]		ND[i]	ND[i]	
LCM[j] ((*R*)-**3**[k])			4.5 [0.5] (3.7−5.5)	10		27 [0.25] (26−28)	6.0	3.9 [2.0] (2.9−6.2)	>500	>120
phenytoin[l]			9.5 [2.0] (8.1−10)			27 [0.25] (26−28)	2.8	30 [4.0] (22−39)	>3000	>100
phenobarbital[l]			22 [1.0] (15−23)			66 [0.5] (63−73)	3.0	9.1 [5.0] (7.6−12)	61 [0.5] (44−96)	6.7
valproate[l]			270 [0.5] (250−340)			430 [0.25] (370−450)	1.6	490 [0.5] (350−730)	280 [0.5] (190−350)	0.6

[a] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. [b] The compounds were administered orally to adult male albino Sprague−Dawley rats. ED_{50} and TD_{50} values are in mg/kg. A dose−response curve was generated for all compounds that displayed sufficient activity (4−6 doses tested, $n = 8$ per dose) and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [c] MES = maximal electroshock seizure test. [d] 6 Hz test = psychomotor seizure model (44 mA). [e] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [f] PI = protective index (TD_{50}/ED_{50}). [g] Tox = behavioral toxicity. [h] Reference 7. [i] ND = not determined. [j] LCM = lacosamide. [k] Reference 12. [l] Reference 46.

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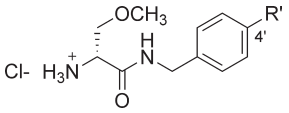
Table 2. Pharmacological Activities of 4′-*N*′-Benzylamide-Substituted C(3)-*O*-Methoxy PAADs (Series 2) in Mice (mg/kg)[a]



compd	R′	MES,[b] ED_{50}	6 Hz,[c] ED_{50}	Formalin, ED_{50}	Tox,[d] TD_{50}	PI,[e] MES	PI,[e] Formalin
(*R*)-**12**	H	34	>67	>67	>120	>3.5	
(*R*)-**24**	Br	31	>180	70	115	3.7	1.6
(*R*)-**25**	CF_3	19	55	>31	48	2.5	
(*R*)-**26**	OCF_3	>10, <30	ND[f]	ND[f]	>30, <100		
(*R*)-**27**	C_6H_5	15	59 (MAD)[g]	>33	ND[f]		
LCM[h] ((*R*)-**3**[i])		3.3	10	15	19	5.8	1.3

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. ED_{50} and TD_{50} values are in mg/kg (4−6 doses tested, $n = 10$ per dose) and were determined 30 min after ip administration. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] ND = not determined. [g] MAD = minimal active dose. [h] LCM = lacosamide. [i] Reference 7.

The neurological activities for 4′-*N*′-benzylamide-substituted C(3)-*O*-methoxy PAADs (*R*)-**24**−**27** (series 2) in mice are listed in Table 2. We systematically evaluated the effect of a bromo, trifluoromethyl, trifluoromethoxy, and phenyl group placed at the 4′-*N*′-benzylamide position on anticonvulsant activity and pain attenuation. The MES activities of all 4′-*N*′-benzylamide-substituted C(3)-*O*-methoxy PAADs increased over the parent, unsubstituted PAAD (*R*)-**12** (ED_{50} = 34 mg/kg). The 4′-phenyl derivative (*R*)-**27** (ED_{50} = 15 mg/kg) and the 4′-trifluoromethyl derivative (*R*)-**25** (ED_{50} = 19 mg/kg) displayed the highest MES activities, a ∼2-fold improvement from (*R*)-**12**. However, the 4′-bromo derivative (*R*)-**24** (ED_{50} = 31 mg/kg) showed marginally improved anticonvulsant activity. While PAADs (*R*)-**24**−**27** displayed significant activity in the MES test, they were 2−6-fold less sensitive to the 6 Hz test. This result is not surprising because a similar decrease in sensitivity (∼3-fold) was observed for (*R*)-**3** (MES ED_{50} = 3.3 mg/kg, 6 Hz ED_{50} = 10 mg/kg),[49] and a >2-fold decrease in sensitivity was observed for (*R*)-**12** (MES ED_{50} = 34 mg/kg, 6 Hz ED_{50} >67 mg/kg).[7] None of the 4′-*N*′-benzylamide-substituted C(3)-*O*-methoxy PAADs displayed appreciable activity in the formalin test at the tested doses. Comparing MES activities of (*R*)-**24**−**27** with their corresponding FAAs[22] revealed a consistent drop in activity (2−10-fold) as we went from FAA to PAAD (Supporting Information Table S2). This drop was similar to the activity drop observed for the unsubstituted *N*′-benzylamide C(3)-alkoxy PAADs and FAAs.[7] Therefore, we concluded that placement of a small substituent at the 4′-*N*′-benzylamide position of C(3)-*O*-methoxy PAADs moderately improved the anticonvulsant activity of PAADs in the MES test but does not provide an advantage over C(2)-hydrocarbon PAADs or FAAs.

Next, we evaluated the neurological activities of 4′-*N*′-benzylamide chimeric PAADs (*R*)-**28**−**34** (series 3 and 4) in mice (Table 3) and rats (Table 4). The data was first analyzed from the standpoint of R, allowing us to identify the optimal R substituent, irrespective of the R′ unit. Then, we examined the effect of R′ on activity; we kept R the same, permitting us to see if there was a preference in the ether linkage orientation of R′ (-OCH_2- versus -CH_2O-). Finally, we evaluated the stereochemical preference for 4′-*N*′-benzylamide chimeric PAADs **30** and **34**.

Using this approach, we determined how introducing either a 3-fluorobenzyloxy or a 3-fluorophenoxymethyl moiety at the 4′-*N*′-benzylamide position (R′) in C(2)-methyl, C(2)-isopropyl, C(2)-*tert*-butyl (series 3), or C(3)-*O*-methoxy (series 4) PAADs affected pharmacological activity. When R′ was 3-fluorobenzyloxy, we observed that most R moieties provided compounds with excellent anticonvulsant activity in the MES test, and the C(2)-*tert*-butyl displayed diminished activity (i.e., C(2)-methyl (*R*)-**28**, ED_{50} = 17 mg/kg; C(2)-isopropyl (*R*)-**29**, ED_{50} = 12 mg/kg; C(2)-*tert*-butyl (*R*)-**31**, ED_{50} = >30, <100 mg/kg; C(2)-CH_2OCH_3 (*R*)-**33**, ED_{50} = 15 mg/kg) (Table 3). Similarly, when R′ was 3-fluorophenoxymethyl, we observed decreased MES activity for the C(2)-*tert*-butyl (*R*)-**32** (ED_{50} = >30, <100 mg/kg), while the C(2)-isopropyl (*R*)-**30** (ED_{50} = 18 mg/kg) and C(2)-CH_2OCH_3 (*R*)-**34** (ED_{50} = ∼10 mg/kg) exhibited potent anticonvulsant activity. This general pattern for the R group was observed when the compounds were tested in the rat (po) (Table 4). The C(2)-*tert*-butyl derivatives (*R*)-**31** and (*R*)-**32** exhibited no or modest MES activity in rats at the doses tested (ED_{50} (mg/kg): (*R*)-**31**, >30; (*R*)-**32**, ∼30), while for a C(2)-CH_2OCH_3 group we saw excellent activity, irrespective of the ether orientation in the R′ unit (ED_{50} (mg/kg): (*R*)-**33**, 13; (*R*)-**34**, 12).

The near equivalence in anticonvulsant activity of the C(2)-isopropyl ((*R*)-**29**, (*R*)-**30**) and C(3)-*O*-methoxy ((*R*)-**33**, (*R*)-**34**) groups is unique to chimeric PAADs. In the unsubstituted PAADs (*R*)-**10** and (*R*)-**12**, we observed that C(2)-isopropyl (*R*)-**10** was 2.3-fold more active than C(3)-*O*-methoxy (*R*)-**12** (ED_{50} (mg/kg): (*R*)-**10**, 15; (*R*)-**12**, 34) (Tables 1 and 2). This activity difference was even greater in the corresponding FAA chimeric series, wherein the terminal amine in the PAAD had been acetylated (Table 5). Here, the C(2)-isopropyl derivative (*R*)-**74** was inactive, while the C(3)-*O*-methoxy compound (*R*)-**76** displayed excellent anticonvulsant activity (ED_{50} (mg/kg): (*R*)-**74**, >300; (*R*)-**76**, 13). The comparable activity of the C(2)-isopropyl ((*R*)-**29**, (*R*)-**30**) and C(3)-*O*-methoxy ((*R*)-**33**, (*R*)-**34**) chimeric PAADs may be due to a DML effect, where adding either a 3-fluorobenzyloxy or a 3-fluorophenoxymethyl pharmacophore at the 4′-*N*-benzylamide position on the (*R*)-**12** PAAD backbone significantly improved the PAAD anticonvulsant activity. A similar but smaller boost was observed for C(2)-isopropyl PAAD (*R*)-**10** when these pharmacophores were included to give PAADs (*R*)-**29** and (*R*)-**30** (Table 3).

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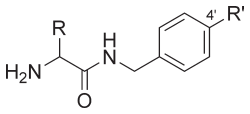
Table 3. Pharmacological Activity of 4′-*N*′-Benzylamide Chimeric PAADs (Series 3 and 4) in Mice (mg/kg)[a]



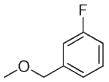
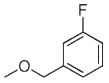
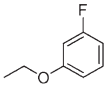
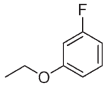
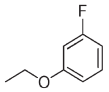
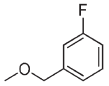
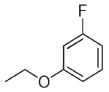
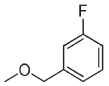
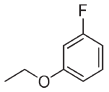
Cmpd No.	Test Site	R	R'	MES,[b] ED_{50}	6 Hz,[c] ED_{50}	Formalin, ED_{50}	Tox,[d] TD_{50}	PI,[e]
(*R*)-**72**	NINDS	CH_3	H	>10, <30	ND[f]	69	>100, <300	
(*S*)-**72**	NINDS	CH_3	H	>300	ND[f]	ND[f]	>300	
(*R*)-**28**	UCB	CH_3		17	>120	>37	ND[f]	
(*R*)-**10**	UCB	$CH(CH_3)_2$	H	16[g] (MAD)	74	20	47	2.9
(*R*)-**10**	NINDS	$CH(CH_3)_2$	H	15 [0.25] (13–18)	<100 [0.25–1.0]	15 [0.25]	70 [0.25] (63–80)	4.7
(*S*)-**10**	NINDS	$CH(CH_3)_2$	H	>300	ND[f]	ND[f]	>300	
(*R*)-**29**	UCB	$CH(CH_3)_2$		12	>110	110[h] (80%)	ND[f]	
(*R*)-**30**	UCB	$CH(CH_3)_2$		12	>110	30	ND[f]	
(*R*)-**30**	NINDS	$CH(CH_3)_2$		18 [0.25] (14–22)	~30 [0.5–1.0]	ND[f]	71 [0.5] (67–78)	3.9
(*S*)-**30**	NINDS	$CH(CH_3)_2$		>30, <100	ND[f]	ND[f]	>100, <300	
(*R*)-**11**	UCB	$C(CH_3)_3$	H	13	>71	>22	ND[f]	
(*R*)-**11**	NINDS	$C(CH_3)_3$	H	14 [0.25] (11–17)	~30 [0.25–0.5]	ND[f]	66 [0.25] (58–73)	4.7
(*S*)-**11**	NINDS	$C(CH_3)_3$	H	42 [0.25] (37–46)	ND[f]	ND[f]	105 [0.25] (100–110)	2.5
(*R*)-**31**	NINDS	$C(CH_3)_3$		>30, <100 [0.5]	ND[f]	ND[f]	~300 [0.5]	
(*R*)-**32**	NINDS	$C(CH_3)_3$		>30, <100 [0.5]	>75	ND[f]	>100, <300 [0.5]	
(*R*)-**12**	UCB	CH_2OCH_3	H	34	>67	>67	>120	>3.5
(*R*)-**12**	NINDS	CH_2OCH_3	H	48 [0.25] (40–61)	ND[f]	ND[f]	>30, <100 [0.25]	
(*S*)-**12**	UCB	CH_2OCH_3	H	64	>70	120	63	1.0
(*R*)-**33**	NINDS	CH_2OCH_3		15 [0.5] (13–17)	<50 [0.5]	ND[f]	58 [0.25] (53–62)	3.9
(*R*)-**34**	UCB	CH_2OCH_3		8.9	58	12 (inactive) 37[h] (94%)	46	5.4

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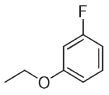
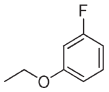
Table 3. Continued

Cmpd No.	Test Site	R	R'	MES,[b] ED_{50}	6 Hz,[c] ED_{50}	Formalin, ED_{50}	Tox,[d] TD_{50}	PI,[e]
(*R*)-**34**	NINDS	CH_2OCH_3		~10 [0.5]	<30	ND[f]	>30, <100 [0.5]	
(*S*)-**34**	NINDS	CH_2OCH_3		>30, <100	ND[f]	ND[f]	>100, <300	

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg (4−6 doses tested, n = 10 per dose) and were determined 30 min after ip administration (UCB) or a dose−response curve was generated for all compounds that displayed sufficient activity (4−6 doses tested, n = 8 per dose) and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] ND = not determined. [g] MAD = minimal active dose. [h] Single dose experiments where the mg/kg used is followed by the percentage protected in parentheses.

Lastly, we examined the C(2)-isopropyl, C(2)-*tert*-butyl, and C(3)-*O*-methoxy PAADs that contained either a 3-fluorobenzyloxy or a 3-fluorophenoxymethyl moiety (R′) to determine if there was a preferred ether linker orientation between the two aromatic rings. Each R set (C(2)-isopropyl, C(2)-*tert*-butyl, C(2)-CH_2OCH_3) displayed similar MES activities in mice for the two ether linkages (-OCH_2- ED_{50} (mg/kg): (*R*)-**29**, 12; (*R*)-**31**, >30, <100; and (*R*)-**33**, 15; and -CH_2O- ED_{50} (mg/kg): (*R*)-**30**, 12; (*R*)-**32**, >30, <100; (*R*)-**34**, 8.9) (Table 3). The formalin activity was evaluated for the C(2)-CH_2OCH_3 PAAD (*R*)-**34** (37 mg/kg (94% reduction)), but the lack of data for (*R*)-**31**, (*R*)-**32**, and (*R*)-**33** prevented any generalized statements. Therefore, on the basis of the available data, both 3-fluorobenzyloxy and 3-fluorophenoxymethyl moieties at the 4′-*N*′-benzylamide position of C(2)-isopropyl ((*R*)-**29** and (*R*)-**30**) and C(2)-CH_2OCH_3 ((*R*)-**33** and (*R*)-**34**) PAADs showed increased MES activity over the corresponding, unsubstituted parent compounds ((*R*)-**10** and (*R*)-**12**).

Comparison of the MES activities of (*R*)-**30** with (*S*)-**30** and (*R*)-**34** with (*S*)-**34** revealed that the higher activity was associated with the (*R*)-isomer (ED_{50} (mg/kg): (*R*)-**30**, 18; (*S*)-**30**, >30, <100; (*R*)-**34**, ~10; (*S*)-**34**, >30, <100). This was not surprising because similar trends were observed in the unsubstituted PAADs (ED_{50} (mg/kg): (*R*)-**72**, >10, <30; (*S*)-**72**, >300; (*R*)-**12**, 34; (*S*)-**12**, 64; (*R*)-**10**, 15; (*S*)-**10**, >300; (*R*)-**11**, 14; (*S*)-**11**, 42)[7] and in the FAA series (ED_{50} (mg/kg): (*R*)-**76**, 13; (*S*)-**76**, >300).[22]

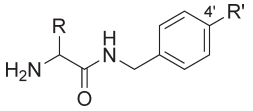
CONCLUSIONS

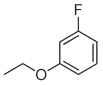
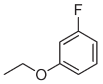
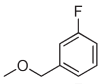
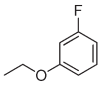
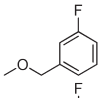
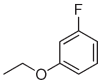
We evaluated 24 C(2)-hydrocarbon and C(3)-*O*-methoxy PAADs that included a 4′-*N*′-benzylamide substituent in whole animal seizure models (MES, 6 Hz psychomotor) and, in some cases, the formalin model of neuropathic pain. The 4′-*N*′-benzylamide substituents included either EW or ED moieties, or structural units found in AAAs (**8**). We discovered that the pharmacological activity of C(2)-hydrocarbon PAADs was highly dependent upon the electronic properties of the 4′-substituent, where 4′-EW groups provided compounds with excellent activity (ED_{50} = <30 mg/kg) and 4′-ED groups provided compounds that were inactive. The correlation between anticonvulsant activity and electronic properties of the 4′-*N*′-benzylamide substituent further distinguished PAADs from FAA (*R*)-**3**, where activity was independent of electronic factors.[22]

Incorporation of the AAA pharmacophore at the 4′-*N*′-benzylamide position of PAADs resulted in potent anticonvulsants, with both the 4′-chimeric C(2)-isopropyl PAAD (*R*)-**30** and C(3)-*O*-methoxy PAAD (*R*)-**34** displaying superb anticonvulsant activity in the MES model (ED_{50} = ≤12 mg/kg). This SAR pattern was unique to PAADs because in the corresponding *N*-acetylated series, the C(2)-hydrocarbon 4′-chimeric derivatives (*R*)-**74** and (*R*)-**75** were devoid of anticonvulsant activity (ED_{50} = >300 mg/kg), while the C(3)-*O*-methoxy 4′-chimeric compounds (*R*)-**76** and (*R*)-**77** exhibited excellent activity (ED_{50} = ≤13 mg/kg).[40] When considering seizure protection, toxicity, the protective indices, and the amount of compound administered in μmol/kg, chimeric PAAD (*R*)-**34** displayed superior anticonvulsant activity (MES ED_{50} = 8.9 mg/kg; UCB value), which approached the therapeutic capabilities of the clinical AED (*R*)-**3** (MES ED_{50} = 3.3 mg/kg). Using the conventional mg/kg dose, it was 2.7-fold less active than (*R*)-**3**, but it exhibited nearly the same PI value as (*R*)-**3** (PI: (*R*)-**34**, 5.4; (*R*)-**3**, 5.8). When the ED_{50} values are converted to μmol/kg, the activity difference between (*R*)-**34** and (*R*)-**3** reduced to 2.0-fold (~25% increase).

The sensitivity of the C(2)-hydrocarbon PAADs to the electronic properties of the 4′-*N*′-benzylamide substituent provided an important difference in the SAR of this series of compounds compared with the FAAs. Additionally, the excellent anticonvulsant activity observed for both the C(2)-hydrocarbon and C(3)-*O*-methoxy chimeric PAADs also differientates this SAR from that of the FAAs. We recognize that the observed differences in the PAAD and FAA SAR may be attributed to changes in pharmacokinetic parameters (e.g., bioavailability, metabolism), but these findings, along with the earlier observation[7] that the anticonvulsant activity for hydrocarbon PAADs did not improve when a substituted heteroatom was included one atom removed from the C(2)-center, suggests that the C(2)-hydrocarbon PAADs have a unique SAR and possibly a mechanism(s) of action that differs from FAAs and other PAADs.

Table 4. Pharmacological Activity of 4′-*N*′-Benzylamide Chimeric PAADs (Series 3 and 4) in Rats (mg/kg)[a]



Cmpd No.	R	R'	MES,[b] ED_{50}	Tox,[c] TD_{50}	PI[d]
(*R*)-**10**	$CH(CH_3)_2$	H	11 [0.25] (9.1–13)	>500	>45
(*R*)-**30**	$CH(CH_3)_2$		<30 [0.5–4.0]	>30 [0.25–4.0]	
(*S*)-**30**	$CH(CH_3)_2$		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**11**	$C(CH_3)_3$	H	11 [0.25] (8.7–15)	>500	>45
(*R*)-**31**	$C(CH_3)_3$		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**32**	$C(CH_3)_3$		~30 [2.0–4.0]	>30 [0.25–4.0]	
(*R*)-**12**	CH_2OCH_3	H	18 [4.0]	>500 [4.0]	>28
(*R*)-**33**	CH_2OCH_3		13 [0.5] (10–18)	>500	>38
(*R*)-**34**	CH_2OCH_3		12 [0.5] (8.2–18)	>500	>42

[a] The compounds were administered orally to adult male albino Sprague–Dawley rats under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg. A dose–response curve was generated for all compounds that displayed sufficient activity (4–6 doses tested, n = 8 per dose), and the dose–effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = behavioral toxicity. [d] PI = protective index (TD_{50}/ED_{50}).

EXPERIMENTAL SECTION

General Methods. The general methods used in this study were identical to those previously reported[7] and are summarized in the Supporting Information. All compounds were checked by TLC, ^1H and ^{13}C NMR, MS, and elemental analyses. The analytical results were within ±0.40% of the theoretical value. The TLC, NMR, and the analytical data confirmed the purity of the products was ≥95%.

General Procedure for PAAD Preparation Using TFA Deprotection (Method A). TFA (15 equiv) was added to an anhydrous CH_2Cl_2 solution of the *t*Boc-protected *N*′-benzylamide PAAD (0.3 M) at room temperature. The solution was stirred (1 h), and then the solvent was evaporated in vacuo. The crude product was subjected to acidic workup or basic workup. Acidic: The crude product was diluted with CH_2Cl_2 and extracted with aqueous 1 M HCl (3×). The combined aqueous layers were washed with CH_2Cl_2 (2×), basified (pH 10–12) with aqueous 4 M NaOH, and extracted with CH_2Cl_2 (3×). The combined organic layers were washed with brine (2×), dried (Na_2SO_4), evaporated in vacuo, and purified by column chromatography (SiO_2). Basic: The crude product was diluted with CH_2Cl_2 and washed with aqueous 1 M Na_2CO_3 (3×). The aqueous layers were combined and washed with CH_2Cl_2 (2×). All of the CH_2Cl_2 layers were combined and successively washed with H_2O (2×) and brine (2×), dried (Na_2SO_4), evaporated in vacuo, and purified by column chromatography (SiO_2).

(*R*)-*N*′-4′-Fluorobenzyl 2-Amino-3-methylbutanamide ((*R*)-13). Utilizing Method A with (*R*)-**44** (4.50 g, 13.9 mmol), TFA (15.5 mL, 209 mmol), and CH_2Cl_2 (46 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.73 g, 87%) as a white solid: mp 86–87 °C; $[\alpha]^{25}_D$ +32.9° (*c* 1.1, $CHCl_3$); R_f 0.47 (1:20 MeOH/CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 1.35 (s, 2H), 2.29–2.41 (m, 1H), 3.27 (d, J = 3.2 Hz, 1H), 4.39 (dd, J = 6.0, 15.0 Hz, 1H), 4.45 (dd, J = 6.0, 14.8 Hz, 1H), 6.97–7.03 (m, 2H), 7.23–7.27 (m, 2H), 7.64–7.72 (br t, 1H); LRMS (ESI) 225.13 [M + H^+] (calcd for $C_{12}H_{17}FN_2OH^+$ 225.13); Anal. ($C_{12}H_{17}FN_2O$): C, H, F, N.

(*R*)-*N*′-4′-Chlorobenzyl 2-Amino-3-methylbutanamide ((*R*)-14). Utilizing Method A with (*R*)-**45** (2.86 g, 8.41 mmol), TFA (6.09 mL, 82.0 mmol), and CH_2Cl_2 (18 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20–1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.52 g, 76%) as a white solid: mp 72–73 °C; $[\alpha]^{28.5}_D$ +26.7° (*c* 1.1, $CHCl_3$); R_f 0.26 (1:20 MeOH/CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, J = 7.2 Hz, 3H), 0.99 (d, J = 6.4 Hz, 3H), 1.30 (s, 2H), 2.28–2.37 (m, 1H), 3.27 (d, J = 3.6 Hz, 1H), 4.37 (dd, J = 6.0, 14.8 Hz, 1H), 4.43 (dd, J = 6.0, 14.8 Hz, 1H), 7.21 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.72–7.80 (br t, 1H); LRMS (ESI) 241.12 [M + H^+] (calcd for $C_{12}H_{17}ClN_2OH^+$ 241.12); Anal. ($C_{12}H_{17}ClN_2O$): C, H, Cl, N.

(*R*)-*N*′-4′-Methylbenzyl 2-Amino-3-methylbutanamide ((*R*)-15). Utilizing Method A with (*R*)-**46** (3.50 g, 10.9 mmol), TFA (12.2 mL, 164 mmol), and CH_2Cl_2 (36 mL) gave the crude

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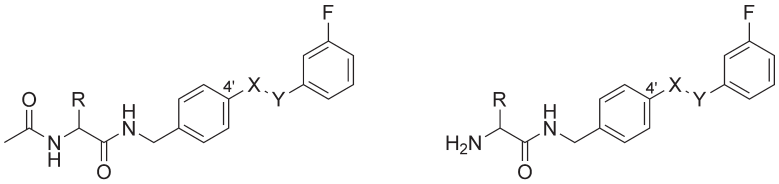
Table 5. Comparison of the Pharmacological Activities of 4′-*N*′-Benzylamide Chimeric FAAs and Their PAAD Counterparts in Mice (mg/kg)[a]



R	X	Y	FAA compd no.	FAA MES,[b] ED_{50}	FAA Tox,[c] TD_{50}	PAAD compd no.	PAAD MES,[b] ED_{50}	PAAD Tox,[c] TD_{50}
CH_3	O	CH_2	(*R*)-**73**[d,e]	>30, <100	>300	(*R*)-**28**[f]	17	ND[g]
$CH(CH_3)_2$	O	CH_2	(*R*)-**74**[d,e]	>300	>300	(*R*)-**29**[f]	12	ND[g]
$C(CH_3)_3$	O	CH_2	(*R*)-**75**[d,e]	>300	>300	(*R*)-**31**[e]	>30, <100 [0.5]	~300 [0.5]
CH_2OCH_3	O	CH_2	(*R*)-**76**[d,e]	13 [0.25] (11−16)	26 [0.5] (21−34)	(*R*)-**33**[e]	15 [0.5] (13−17)	58 [0.25] (53−62)
CH_2OCH_3	O	CH_2	(*S*)-**76**[d,e]	>300	>300	(*S*)-**33**[e]	ND[g]	ND[g]
CH_2OCH_3	CH_2	O	(*R*)-**77**[d,e]	5.9 [0.25] (4.3−7.3)	10 [0.25] (9.1−13)	(*R*)-**34**[f]	8.9	46
CH_2OCH_3	CH_2	O	(*S*)-**77**[d,e]	ND[g]	ND[g]	(*S*)-**34**[e]	>30, <100	>100, <300

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg (4−6 doses tested, n = 10 per dose) and were determined 30 min after ip administration (UCB) or a dose−response curve was generated for all compounds that displayed sufficient activity (4−6 doses tested, n = 8 per dose), and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Reference 40. [e] The compounds were administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. [f] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB. [g] ND = not determined.

product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.70 g, 71%) as a white solid: mp 67−68 °C; $[\alpha]^{28.5}_D$ +26.7° (*c* 1.1, $CHCl_3$); R_f 0.53 (1:10 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H), 1.33 (s, 2H), 2.29−2.40 (m, 1H), 2.33 (s, 3H), 3.27 (d, J = 4.0 Hz, 1H), 4.38 (dd, J = 6.0, 14.8 Hz, 1H), 4.44 (dd, J = 6.0, 14.8 Hz, 1H), 7.13 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 7.54−7.62 (br t, 1H); HRMS (ESI) 221.1664 [M + H$^+$] (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654); Anal. ($C_{13}H_{20}N_2O$•0.05H_2O): C, H, N.

(*R*)-*N*′-4′-(Trifluoromethyl)benzyl 2-Amino-3-methylbutanamide ((*R*)-16). Utilizing Method A with (*R*)-**47** (4.00 g, 10.7 mmol), TFA (11.9 mL, 161 mmol), and CH_2Cl_2 (35 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:10−1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.18 g, 75%) as a white solid: mp 86−87 °C; $[\alpha]^{28.5}_D$ +26.0° (*c* 1.0, $CHCl_3$); R_f 0.37 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 7.2 Hz, 3H), 1.00 (d, J = 7.2 Hz, 3H), 1.32 (s, 2H), 2.30−2.41 (m, 1H), 3.30 (d, J = 4.0 Hz, 1H), 4.47 (dd, J = 6.0, 15.4 Hz, 1H), 4.53 (dd, J = 6.0, 15.4 Hz, 1H), 7.39 (d, J = 7.6 Hz, 2H), 7.57 (d, J = 7.6 Hz, 2H), 7.84−7.92 (br t, 1H); LRMS (ESI) 275.14 [M + H$^+$] (calcd for $C_{13}H_{17}F_3N_2OH^+$ 275.14); Anal. ($C_{13}H_{17}F_3N_2O$): C, H, F, N.

(*R*)-*N*′-4′-Methoxybenzyl 2-Amino-3-methylbutanamide ((*R*)-17). Utilizing Method A with (*R*)-**48** (4.50 g, 13.4 mmol), TFA (14.9 mL, 201 mmol), and CH_2Cl_2 (45 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (3.04 g, 96%) as a white solid: mp 81−82 °C; $[\alpha]^{28.5}_D$ +25.5° (*c* 1.1, $CHCl_3$); R_f 0.42 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H), 1.33 (s, 2H), 2.28−2.40 (m, 1H), 3.26 (d, J = 3.6 Hz, 1H), 3.79 (s, 3H), 4.36 (dd, J = 5.6, 14.6 Hz, 1H), 4.41 (dd, J = 6.0, 14.6 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 7.52−7.58 (br t, 1H); LRMS (ESI) 237.17 [M + H$^+$] (calcd for $C_{13}H_{20}N_2O_2H^+$ 237.17); Anal. ($C_{13}H_{20}N_2O_2$): C, H, N.

(*R*)-*N*′-4′-(Trifluoromethoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-18). Utilizing Method A with (*R*)-**49** (3.20 g, 8.20 mmol), TFA (9.14 mL, 123 mmol), and CH_2Cl_2 (27 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:20−1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.33 g, 56%) as a pale yellow solid: mp 63−64 °C; $[\alpha]^{28.5}_D$ +26.6° (*c* 1.0, $CHCl_3$); R_f 0.47 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 7.2 Hz, 3H), 1.00 (d, J = 7.2 Hz, 3H), 1.36 (s, 2H), 2.33−2.41 (m, 1H), 3.30 (d, J = 3.2 Hz, 1H), 4.40−4.51 (m, 2H), 7.17 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.70−7.80 (br t, 1H); LRMS (ESI) 291.15 [M + H$^+$] (calcd for $C_{13}H_{17}F_3N_2O_2H^+$ 291.15); Anal. ($C_{13}H_{17}F_3N_2O_2$): C, H, F, N.

(*R*)-*N*′-(Biphenyl-4′-yl)methyl 2-Amino-3-methylbutanamide ((*R*)-19).[50] Utilizing Method A with (*R*)-**50** (2.51 g, 6.57 mmol), TFA (7.32 mL, 98.5 mmol), and CH_2Cl_2 (22 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10−1:1 EtOAc/hexanes) to give the desired compound (1.81 g, 98%) as a white solid: mp 96−97 °C; $[\alpha]^{25}_D$ +16.5° (*c* 1.0, CH_2Cl_2); R_f 0.28 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (d, J = 7.0 Hz, 3H), 1.01 (d, J = 7.0 Hz, 3H), 1.45 (s, 2H), 2.33−2.42 (m, 1H), 3.32 (d, J = 3.2 Hz, 1H), 4.45−4.55 (m, 2H), 7.32−7.37 (m, 3H), 7.43 (m, 2H), 7.56 (m, 4H), 7.62−7.68 (br t, 1H); HRMS (ESI) 283.1800 [M + H$^+$] (calcd for $C_{18}H_{22}N_2OH^+$ 283.1810); Anal. ($C_{18}H_{22}N_2O$•0.07CH_2Cl_2): C, H, N.

(*R*)-*N*′-4′-Chlorobenzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-20). Utilizing Method A with (*R*)-**51** (2.50 g, 7.06 mmol), TFA (7.86 mL, 106 mmol), and CH_2Cl_2 (23 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (1.31 g, 73%) as a white solid: mp 78−79 °C; $[\alpha]^{25}_D$ +14.2° (*c* 1.0, CH_2Cl_2); R_f 0.23 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (s, 9H), 1.47 (s, 2H), 3.14 (s, 1H), 4.36 (1/2 AB_q, J = 17.6 Hz, 1H),

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4.43 (1/2 AB_q, J = 17.6 Hz, 1H), 7.22 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 8.6 Hz, 2H); HRMS (ESI) 255.1256 [M + H^+] (calcd for $C_{13}H_{19}ClN_2O$-H^+ 255.1264); Anal. ($C_{13}H_{19}ClN_2O$): C, H, Cl, N.

(*R*)-*N*′-4′-(Trifluoromethyl)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-21). Utilizing Method A with (*R*)-**52** (2.50 g, 6.44 mmol), TFA (7.18 mL, 96.6 mmol), and CH_2Cl_2 (21 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:10−1:1 EtOAc/hexanes) to give the desired compound (0.68 g, 37%) as a white solid: mp 90−91 °C; $[\alpha]^{25}_D$ +17.2° (c 1.0, CH_2Cl_2); R_f 0.14 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 9H), 1.49 (s, 2H), 3.17 (s, 1H), 4.44−4.54 (m, 2H), 7.29−7.37 (br t, 1H), 7.40 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 7.6 Hz, 2H); HRMS (+ESI) 289.1515 [M + H^+] (calcd. for $C_{14}H_{19}F_3N_2OH^+$ 289.1528); Anal. ($C_{14}H_{19}F_3N_2O$): C, H, F, N.

(*R*)-*N*′-4′-(Trifluoromethoxy)benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-22). Utilizing Method A with (*R*)-**53** (2.00 g, 4.95 mmol), TFA (5.51 mL, 74.2 mmol), and CH_2Cl_2 (16 mL) gave the crude product after acidic workup that was further purified by flash column chromatography (SiO_2; 1:10−1:1 EtOAc/hexanes) to give the desired compound (0.93 g, 62%) as a white solid: mp 68−69 °C; $[\alpha]^{25}_D$ +14.7° (c 1.1, CH_2Cl_2); R_f 0.29 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 9H), 1.56 (s, 2H), 3.14 (s, 1H), 4.43 (d, J = 6.0 Hz, 2H), 7.17 (d, J = 8.6 Hz, 2H), 7.23−7.29 (br s, 1H), 7.31 (d, J = 8.6 Hz, 2H); HRMS (ESI) 305.1463 [M + H^+] (calcd for $C_{14}H_{19}F_3N_2O_2H^+$ 305.1477); Anal. ($C_{14}H_{19}F_3N_2O_2$): C, H, F, N.

(*R*)-*N*′-(Biphenyl-4′-yl)methyl 2-Amino-3,3-dimethylbutanamide ((*R*)-23). Utilizing Method A with (*R*)-**54** (1.53 g, 3.86 mmol), TFA (4.30 mL, 57.9 mmol), and CH_2Cl_2 (13 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10−1:1 EtOAc/hexanes) to give the desired compound (0.76 g, 67%) as a white solid: mp 75−76 °C; $[\alpha]^{28.5}_D$ +14.4° (c 1.0, CH_2Cl_2); R_f 0.50 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 9H), 1.50 (s, 2H), 3.16 (s, 1H), 4.44−4.54 (m, 2H), 7.06−7.12 (br s, 1H), 7.32−7.37 (m, 3H), 7.42−7.45 (m, 2H), 7.55−7.59 (m, 4H); LRMS (ESI) 297.20 [M + H^+] (calcd for $C_{19}H_{24}N_2OH^+$ 297.20); Anal. ($C_{19}H_{24}N_2O$): C, H, N.

(*R*)-*N*′-4′-Bromobenzyl 2-Amino-3-methoxypropionamide Hydrochloride ((*R*)-24). HCl (4.0 M in dioxane, 16 mL) was added at 0 °C to (*R*)-**55**[22] (2.24 g, 5.78 mmol), and the solution was stirred at room temperature overnight. The reaction was concentrated in vacuo and triturated with Et_2O to give the desired compound (1.18 g, 63%) as a white solid: mp 165−167 °C; $[\alpha]^{25}_D$ +1.2° (c 1, MeOH); R_f 0.13 (EtOAc); ^{1}H NMR (DMSO-d_6) δ 3.30 (s, 3H), 3.67−3.77 (m, 2H), 4.07 (t, J = 4.8 Hz, 1H), 4.25−4.39 (m, 2H), 7.25 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 8.26−8.42 (br s, 3H), 9.20 (t, J = 5.7 Hz, 1H); LRMS (ESI) 287.0 [M + H^+] (100%), 289.1 [M + 2 + H^+] (100%) (calcd for $C_{11}H_{15}{}^{79}BrN_2O_2H^+$ 287.0); Anal. ($C_{11}H_{16}ClBrN_2O_2$): C, H, Br, Cl, N.

(*R*)-*N*′-4′-(Trifluoromethyl)benzyl 2-Amino-3-methoxypropionamide Hydrochloride ((*R*)-25). HCl (4.0 M in dioxane, 16 mL) was added at 0 °C to (*R*)-**56**[22] (3.00 g, 7.98 mmol), and the solution was stirred at room temperature overnight. The reaction was concentrated in vacuo and triturated with Et_2O to give the desired compound (2.28 g, 91%) as a white solid: mp 181 °C; $[\alpha]^{24.5}_D$ −5.14° (c 1, MeOH); R_f 0.08 (EtOAc); ^{1}H NMR (DMSO-d_6) δ 3.32 (s, 3H), 3.73−3.75 (m, 2H), 4.08−4.11 (m, 1H), 4.37−4.51 (m, 2H), 7.51 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H), 8.32 (br s, 3H), 9.28 (br s, 1H); HRMS (ESI) 277.1164 [M + H^+] (calcd for $C_{12}H_{15}F_3N_2O_2H^+$ 277.1158); Anal. ($C_{12}H_{16}ClF_3N_2O_2$): C, H, Cl, F, N.

(*R*)-*N*′-4′-(Trifluoromethoxy)benzyl 2-Amino-3-methoxypropionamide Hydrochloride ((*R*)-26). An EtOH solution (100 mL) of (*R*)-**57**[22] (1.00 g, 2.65 mmol) was treated with H_2 (1 atm) in presence of 10% Pd−C (100 mg) at room temperature (16 h). The mixture was carefully filtered through a bed of Celite, and the filtrate was evaporated in vacuo to obtain a yellow solid. The solid was dissolved in Et_2O (10 mL) and HCl (1.0 M in Et_2O, 5 mL) was added dropwise. The resulting mixture was stirred (5 min), and the white precipitate was filtered and dried to give the desired compound as a white solid: mp 169−170 °C; $[\alpha]^{25}_D$ −4.7° (c 1, MeOH); R_f 0.08 (EtOAc); ^{1}H NMR (DMSO-d_6) δ 3.31 (s, 3H), 3.72−3.74 (m, 2H), 4.01−4.12 (br s, 1H), 4.31−4.44 (m, 2H), 7.34 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 8.26−8.44 (br s, 3H), 9.19−9.23 (app. t, 1H); HRMS (ESI) 293.1105 [M + H^+] (calcd for $C_{12}H_{15}F_3N_2O_3H^+$ 293.1108); Anal. ($C_{12}H_{16}$-$ClF_3N_2O_3$): C, H, Cl, F, N.

(*R*)-*N*′-(Biphenyl-4′-yl)methyl 2-Amino-3-methoxypropionamide Hydrochloride ((*R*)-27). A saturated HCl solution in dioxane (1 mmol/2 mL, 11.5 mL) was added to (*R*)-**58**[22] (2.20 g, 5.73 mmol) at 0 °C, and the solution was stirred at room temperature (12 h). A second saturated HCl solution in dioxane (1 mmol/2 mL, 11.5 mL) was added, and the solution was stirred at room temperature (6 h). The white solid was filtered to give the desired compound (1.25 g, 70%): mp 145−148 °C; $[\alpha]^{27.0}_D$ +33.2° (c 0.5, H_2O); ^{1}H NMR (DMSO-d_6) δ 3.32 (s, 3H), 3.75 (d, J = 4.6 Hz, 2H), 4.09 (t, J = 4.6 Hz, 1H), 4.36−4.44 (m, 2H), 7.34−7.41 (s, 3H), 7.43−7.90 (m, 2H), 7.61−7.68 (m, 4H), 8.31−8.44 (br s, 3H), 9.22 (t, J = 5.7 Hz, 1H); Anal. ($C_{17}H_{21}ClN_2O_2$): C, H, Cl, N.

(*R*)-*N*′-4′-(3″-Fluorobenzyloxy)benzyl 2-Aminopropionamide Hydrochloride ((*R*)-28). HCl in dioxane (4.0 M, 2.4 mL, 4.7 mmol) was added to (*R*)-**63** (1.90 g, 4.72 mmol) at room temperature (2 h). The precipitate was filtered to give the desired compound (1.55 g, 100%) as a white solid: mp 105−107 °C; $[\alpha]^{26}_D$ −11.4° (c 0.5, DMSO); R_f 0.00 (EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.37 (s, J = 6.6 Hz, 3H), 3.80−3.87 (br m, 1H), 5.12 (s, 2H), 6.97 (d, J = 8.1 Hz, 2H), 7.10−7.30 (m, 5H), 7.40−7.45 (m, 1H), 8.17−8.25 (br d, 3H), 8.86−8.92 (br s, 1H); HRMS (ESI) 303.1518 [M + H^+] (calcd for $C_{17}H_{19}$-$FN_2O_2H^+$ 303.1503); Anal. ($C_{17}H_{20}ClFN_2O_2 \cdot 0.10H_2O$): C, H.

(*R*)-*N*′-4′-(3″-Fluorobenzyloxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-29). Utilizing Method A with (*R*)-**64** (2.00 g, 4.65 mmol), TFA (5.18 mL, 69.7 mmol), and CH_2Cl_2 (15 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (481 mg, 32%) as a white solid: mp 77−78 °C; $[\alpha]^{25}_D$ +16.8° (c 1.0, CH_2Cl_2); R_f 0.57 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 7.2 Hz, 3H), 1.31−1.58 (br s, 2H), 2.31−2.38 (m, 1H), 3.26 (d, J = 2.8 Hz, 1H), 4.33−4.44 (m, 2H), 5.05 (s, 2H), 6.91 (d, J = 7.2 Hz, 2H), 7.00 (t, J = 8.4 Hz, 1H), 7.13−7.26 (m, 4H), 7.31−7.39 (m, 1H), 7.51−7.59 (br t, 1H); HRMS (ESI) 331.1827 [M + H^+] (calcd for $C_{19}H_{23}FN_2O_2H^+$ 331.1822); Anal. ($C_{19}H_{23}FN_2O_2$): C, H, F, N.

(*R*)-*N*′-4′-(3″-Fluorophenoxymethyl)benzyl 2-Amino-3-methylbutanamide ((*R*)-30). Utilizing Method A with (*R*)-**65** (3.00 g, 6.97 mmol), TFA (7.77 mL, 105 mmol), and CH_2Cl_2 (23 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (0.92 g, 40%) as a white solid: mp 78−79 °C; $[\alpha]^{25}_D$ +17.5° (c 1.1, CH_2Cl_2); R_f 0.50 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, J = 6.8 Hz, 3H), 1.00 (d, J = 7.2 Hz, 3H), 1.26−1.58 (br s, 2H), 2.33−2.41 (m, 1H), 3.29 (d, J = 3.6 Hz, 1H), 4.41−4.52 (m, 2H), 5.03 (s, 2H), 6.64−6.69 (m, 2H), 6.73−6.76 (m, 1H), 7.12−7.39 (m, 5H), 7.62−7.84 (br t, 1H); HRMS (ESI) 331.1828 [M + H^+] (calcd for $C_{19}H_{23}FN_2O_2H^+$ 331.1822); Anal. ($C_{19}H_{23}FN_2O_2$): C, H, F, N.

(*S*)-*N*′-4′-(3″-Fluorophenoxymethyl)benzyl 2-Amino-3-methylbutanamide ((*S*)-30). The previous procedure was repeated using (*S*)-**65** (4.00 g, 9.30 mmol), TFA (10.4 mL, 139 mmol), and CH_2Cl_2 (30 mL) to give the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired

compound (2.67 g, 87%) as a white solid: mp 75−76 °C; $[\alpha]^{28}_{D}$ −18.2° (*c* 1.2, CH_2Cl_2); R_f 0.45 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 7.0 Hz, 3H), 1.20−1.52 (br s, 2H), 2.34−2.40 (m, 1H), 3.24−3.34 (br d, 1H), 4.41−4.52 (m, 2H), 5.02 (s, 2H), 6.64−6.70 (m, 2H), 6.73−6.76 (m, 1H), 7.12−7.25 (m, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.64−7.70 (br t, 1H); LRMS (ESI) 331.14 [M + H$^+$] (calcd for $C_{19}H_{23}FN_2O_2H^+$ 331.14); Anal. ($C_{19}H_{23}FN_2O_2$): C, H, F, N.

(*R*)-*N*′-4′-(3″-Fluorophenoxymethyl)benzyl 2-Amino-3, 3-dimethylbutanamide ((*R*)-32). Utilizing Method A with (*R*)-**67** (1.82 g, 4.10 mmol), TFA (4.56 mL, 61.5 mmol), and CH_2Cl_2 (14 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:10−1:1 EtOAc/hexanes) to give the desired compound (1.03 g, 73%) as a white solid: mp 59−60 °C; $[\alpha]^{25}_{D}$ +9.3° (*c* 1.1, CH_2Cl_2); R_f 0.30 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 9H), 1.50 (s, 2H), 3.14 (s, 1H), 4.45 (d, *J* = 4.2 Hz, 2H), 5.03 (s, 2H), 6.65−6.69 (m, 2H), 6.75 (d, *J* = 6.0 Hz, 1H), 7.02−7.14 (br t, 1H), 7.19−7.25 (m, 1H), 7.31 (d, *J* = 5.3 Hz, 2H), 7.39 (d, *J* = 5.3 Hz, 2H); HRMS (ESI) 345.1977 [M + H$^+$] (calcd for $C_{20}H_{25}FN_2O_2H^+$ 345.1978); Anal. ($C_{20}H_{25}FN_2O_2$): C, H, F, N.

(*R*)-*N*′-4′-(3″-Fluorobenzyloxy)benzyl 2-Amino-3-methoxypropionamide ((*R*)-33). Utilizing Method A with (*R*)-**70** (3.00 g, 6.94 mmol), TFA (7.73 mL, 104 mmol), and CH_2Cl_2 (23 mL) gave the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (2.05 g, 89%) as a pale yellow solid: mp 76−77 °C; $[\alpha]^{28.5}_{D}$ +7.9° (*c* 1.1, $CHCl_3$); R_f 0.74 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 2H), 3.36 (s, 3H), 3.56−3.64 (m, 3H), 4.33−4.44 (m, 2H), 5.04 (s, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 6.98−7.02 (m, 1H), 7.13−7.21 (m, 4H), 7.34 (q, *J* = 8.0 Hz, 1H), 7.68−7.72 (br t, 1H); LRMS (ESI) 333.17 [M + H$^+$] (calcd for $C_{18}H_{21}FN_2O_3H^+$ 333.17); Anal. ($C_{18}H_{21}FN_2O_3$): C, H, F, N.

(*R*)-*N*′-4′-(3″-Fluorophenoxymethyl)benzyl 2-Amino-3-methoxypropionamide ((*R*)-34). Utilizing Method A with (*R*)-**71** (2.45 g, 5.67 mmol), TFA (6.32 mL, 85.1 mmol), and CH_2Cl_2 (19 mL) gave the crude product after basic workup that was further purified twice by flash column chromatography (SiO_2; 1:20−1:1 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) followed by recrystallization from hot EtOAc/hexanes to give the desired product (1.30 g, 69%) as a pale yellow solid: mp 60−61 °C; $[\alpha]^{25}_{D}$ +6.1° (*c* 1.1, $CHCl_3$); R_f 0.76 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 2H), 3.38 (s, 3H), 3.59−3.65 (m, 3H), 4.42−4.52 (m, 2H), 5.03 (s, 2H), 6.64−6.69 (m, 2H), 6.75 (d, *J* = 9.6 Hz, 1H), 7.22 (q, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.74−7.83 (br t, 1H); LRMS (ESI) 333.17 [M + H$^+$] (calcd for $C_{18}H_{21}FN_2O_3H^+$ 333.17); Anal. ($C_{18}H_{21}FN_2O_3$): C, H, F, N.

(*S*)-*N*′-4′-(3″-Fluorophenoxymethyl)benzyl 2-Amino-3-methoxypropionamide ((*S*)-34). The previous procedure was repeated using (*S*)-**71** (2.71 g, 6.27 mmol), TFA (6.99 mL, 94.1 mmol), and CH_2Cl_2 (21 mL) to give the crude product after basic workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give a mixture of desired product and impurity as an orange oil. The crude oil obtained after the acid workup was then further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (0.27 g, 19%) as a pale yellow solid: mp 52−53 °C; $[\alpha]^{25}_{D}$ −6.8° (*c* 1.1, $CHCl_3$); R_f 0.29 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (s, 2H), 3.38 (s, 3H), 3.59−3.65 (m, 3H), 4.42−4.52 (m, 2H), 5.03 (s, 2H), 6.64−6.70 (m, 2H), 6.73−6.76 (m, 1H), 7.19−7.26 (m, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.76−7.84 (br t, 1H); LRMS (ESI) 333.12 [M + H$^+$] (calcd for $C_{18}H_{21}FN_2O_3H^+$ 333.12); Anal. ($C_{18}H_{21}FN_2O_3$): C, H. F, N.

Pharmacology. Compounds were screened under the auspices of UCB Pharma (Braine L′Alleud, Belgium) and the NINDS ASP (Rockville, MD). Housing, handling, and feeding were in full accordance with recommendations contained in the Guide for the Care and Use of Laboratory Animals.[51] Pharmacological evaluation by UCB Pharma consisted of four assays using male NMRI mice (ip): the 6 Hz test[42] and the MES test[41] to assess anticonvulsant activity, the formalin test[10,43] to assess neuropathic pain attenuation, and the rotorod test[41] to assess neurological toxicity. Pharmacological evaluation by the NINDS ASP utilized male albino Carworth Farms No. 1 mice (ip) or male albino Sprague−Dawley rats (po) and consisted of the MES test (mice and rats) and the subcutaneous pentylenetetrazol (metrazol) (scMet) seizure threshold test[8] to assess anticonvulsant activity (mice), the rotorod test to assess neurological toxicity (mice), and the positional sense test or gait and stance test to assess behavioral toxicity (rats).[8]

■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures and spectral properties (IR, ^{1}H and ^{13}C NMR, MS) for the synthetic intermediates and the PAADs, and tables of elemental analyses (Table S3) and MS spectra (Table S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

AAA, α-aminoamide; AED, antiepileptic drug; ASP, Anticonvulsant Screening Program; CCI, chronic constriction injury; CNS, central nervous system; DMLs, designed multiple ligands; ED, electron-donating; ED_{50}, effective dose (50%); EW, electron-withdrawing; FAA, functionalized amino acid; HCl, hydrochloride; ip, intraperitoneally; MAC, mixed anhydride coupling; MES, maximal electroshock seizure; NINDS, National Institute of Neurological Disorders and Stroke; PAAD, primary amino acid derivative; PI, protective index; po, orally; SAR, structure−activity relationship; scMet, subcutaneous metrazol; TD_{50}, neurological impairment (toxicity, 50%); TFA, trifluoroacetic acid

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