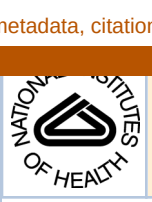

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Defining the Structural Parameters that Confer Anticonvulsant Activity by the Site-by-Site Modification of (*R*)-*N*′-Benzyl 2-Amino-3-methylbutanamide

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Abstract

Primary Amino Acid Derivatives (PAADs) (*N*′-benzyl 2-substituted 2-amino acetamides) are structurally related to Functionalized Amino Acids (FAAs) (*N*′-benzyl 2- substituted 2-acetamido acetamides) but differ by the absence of the terminal *N*-acetyl group. Both classes exhibit potent anticonvulsant activities in the maximal electroshock seizure animal model and the reported structure-activity relationships (SARs) of PAADs and FAAs differ in significant ways. Recently, we documented that PAAD efficacy was associated with a hydrocarbon moiety at the C(2)-carbon, while in the FAAs, a substituted heteroatom one atom removed from the C(2)-center was optimal. Previously in this issue, we showed that PAAD activity was dependent upon the electronic properties of the 4′-*N*′-benzylamide substituent, while FAA activity was insensitive to electronic changes at this site. In this study, we prepared analogs of (*R*)-*N*′-benzyl 2-amino-3-methylbutanamide to identify the structural components for maximal anticonvulsant activity. We demonstrated that the SAR of PAADs and FAAs diverged at the terminal amide site and that PAADs had considerably more structural latitude in the types of units that could be incorporated at this position, suggesting that these compounds function according to different mechanism(s).

INTRODUCTION

Epilepsy is a heterogeneous mixture of disorders characterized by neuronal hyperexcitability and hypersynchronous neuronal firing, and it affects up to 1% of the world's population.[1] There are over 40 antiepileptic drugs (AEDs) currently in clinical use,[2] yet, some 30% of patients are pharmacoresistant and do not respond to at least two of the first-line AEDs.[3] Furthermore, compliance is often limited by adverse side effects (e.g., drowsiness, dizziness, nausea), experienced by nearly 40% of patients.[4] Novel compounds with increased efficacy

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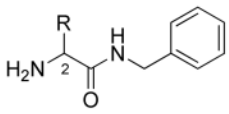
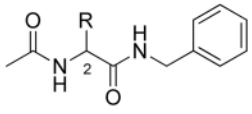
Supporting Information Available: Synthetic procedures and spectral properties (IR, ^{1}H and ^{13}C NMR, MS) for the synthetic intermediates and the PAADs, table of elemental analyses (Supporting Information Table S1) and MS spectra (Supporting Information Table S2). This material is available free of charge via the internet at http://pubs.acs.org.

and decreased toxicity are needed to improve the quality of life of those suffering from epilepsy.

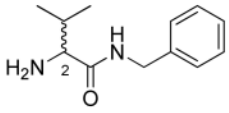
We recently reported that Primary Amino Acid Derivatives (PAADs[a], **1**) exhibit potent anticonvulsant activities in the maximal electroshock seizure (MES) test[5] in rodents,[6] with activities approaching that of many clinical AEDs.[7] Significantly, the structure-activity relationship (SAR) for PAADs and their *N*-acetylated analogs, the Functionalized Amino Acids (FAAs, **2**), differed in several ways. First, PAAD anticonvulsant activity, unlike the FAAs, did not improve when a substituted heteroatom was included one atom removed from the C(2) chiral center.[6] Second, PAAD activity was sensitive to the electronic properties of *para*-substituents on the *N′*-benzylamide ring, where activity was retained with an electron-withdrawing group but lost upon inclusion of an electron-releasing group.[8] By comparison, FAAs containing either electron-donating or electron-withdrawing groups at the *para*-position provided compounds with excellent anticonvulsant activities.[9] Third, the maximal activity for C(2)-hydrocarbon PAADs resided in the D-configuration, similar to the FAAs, but the differences in the ratio of the active stereoisomer to the less-active stereoisomer (eudismic ratio)[10] differed widely. For (*R*)-**3** and (*S*)- **3**, the eudismic ratio in mice exceeded 20, while for (*R*)-**4** and (*S*)-**4** it was 3. For FAAs, the eudismic ratio was consistently above 10.[9, 11, 12] The stark contrast between PAAD and FAA activity was most apparent for C(2)-hydrocarbon derivatives, where the C(2)-hydrocarbon was a branched alkyl moiety. We found that the C(2)-isopropyl PAAD (*R*)-**3** (MES ED_{50} = 15 mg/kg) and C(2)-*tert*-butyl PAAD (*R*)-**4** (MES ED_{50} = 14 mg/kg) were potent anticonvulsants, while their FAA counterparts (*R*)-**5** (MES ED_{50} = >100, <300 mg/kg) and (*R*)-**6** (MES ED_{50} = > 300 mg/kg) were not.[6] Collectively, these results suggest that the C(2)-hydrocarbon PAADs represent a novel class of anticonvulsants.



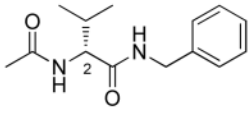
1
PAAD



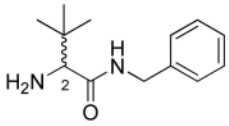
2
FAA



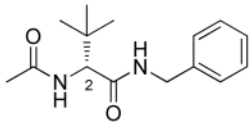
(*R*)-**3** and (*S*)-**3**



(*R*)-**5**



(*R*)-**4** and (*S*)-**4**



(*R*)-**6**

[a]Abbreviations: AED, antiepileptic drug; ASP, Anticonvulsant Screening Program; CMDT, 2-chloro-4,6-dimethoxy-1,3,5-triazine; CNS, central nervous system; DMAP, 4- dimethylaminopyridine; DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; ED_{50}, effective dose (50%); FAA, functionalized amino acid; FAK, functionalized amido ketone; 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; SAAD, secondary amino acid derivative; SAR, structure-activity relationship; scMet, subcutaneous Metrazol®; TD_{50}, neurological impairment (toxicity, 50%); TAAD, tertiary amino acid derivative; TFA, trifluoroacetic acid.

The differences in the PAAD and FAA SAR have led us to question the optimal chemical framework for PAADs. At the outset,[6] we assumed that PAADs and FAAs would require similar structural features for maximal activity. Among these were the diamine backbone containing a carbonyl unit and a *N*′-terminal benzylamide. In the present study, we systematically modified six sites in the core structure of PAAD **3**. Significantly, we found that several key structural motifs associated with maximal FAA seizure protection were not necessary for potent PAAD activity. These findings provided additional evidence that C(2)-hydrocarbon PAADs are a distinct, new class of potent anticonvulsants. The structural simplicity of these PAADs, and the excellent water solubility of their corresponding salts, provide opportunities for further development.

RESULTS AND DISCUSSION

Choice of Compounds

C(2)-Hydrocarbon PAADs possess significant anticonvulsant activities and our studies indicated that many of the FAA structural hallmarks do not apply to PAADs.[6, 8] Therefore, we investigated the originally proposed PAAD structural framework. For most of our studies, we systematically modified (*R*)-C(2)-isopropyl PAAD **3**, which was among the most potent PAADs discovered. Six structural features common to both PAADs and FAAs were examined by preparing **7**–**24** (Figure 1): the carbonyl unit (Site A); the amide bond (Site B); the amide methylene linker length (Site C); the regiosubstitution of the *N*′-benzylamide (Site D); the need for a *N*′-benzylamide (Site E); and the C(2)-amino functionality (Site F). All C(2)- isopropyl PAAD analogs (**7**–**18**, **23**, **24**) were synthesized in the (*R*)-configuration. For *N*′-benzyl 2-aminobutanamide **19** and *N*-benzyl 2-hydroxybutanamide **22**, we synthesized the (*R*)- configuration, for **21**, we prepared the (*R*,*S*) and (*S*)-configurations, and *N*-benzyl butanamide (**20**) does not have a chiral center.

C(2)-Isopropyl PAAD analogs (Sites A–C)

The importance of the carbonyl unit for FAA anticonvulsant activity has been demonstrated by an isoelectronic substitution of the amide carbonyl with a thiocarbonyl group,[12] and we conducted a similar investigation using (*R*)-**7** (Site A). We further examined the value of the carbonyl unit by replacing it with a methylene group ((*R*)-**8**).

We have reported that functionalized amido ketones (FAKs) exhibit significant anticonvulsant activities that were comparable to FAAs; however, an increase in neurological toxicity was also observed (Site B).[13] In a similar manner, we replaced the nitrogen of the amide bond in (*R*)-**3** with a methylene group ((*R*)-**9**) or oxygen ((*R*)-**10**) to create either a primary amino ketone or primary amino ester, respectively.

The distance between the amide bond and the aromatic ring was briefly investigated for FAAs, and maximal activity was seen for the *N*′-benzylamide moiety (n = 1). Therefore, we examined the anticonvulsant activities of PAADs that contain 0–4 methylene units between the amide bond and the aromatic ring ((*R*)-**3**, (*R*)-**11**–**14**) (Site C).

Regiosubstitution in *N*′-Benzylamide PAADs (Site D)

Recently, we systematically evaluated the effect of a fluoro and trifluoromethoxy group at the 2′, 3′, and 4′ postions in the *N*′-benzyl moiety of FAAs and determined that the 4′ derivatives displayed the highest level of anticonvulsant activity,[9] consistent with previous studies.[11, 14] Accordingly, we evaluated the effect of a trifluoromethoxy group at the 2′, 3′, and 4′ positions of PAAD (*R*)-**3** by preparing (*R*)-**15**, (*R*)-**16**, and (*R*)-**17**,[8] respectively.

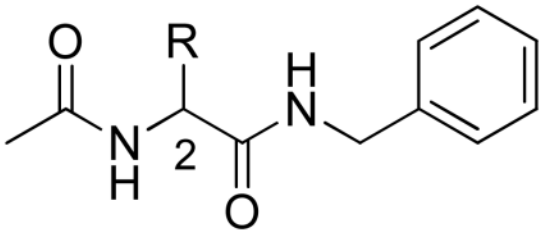
N′-Substituted C(2)-isopropyl PAADs (Site E)

We evaluated several *N*′-substituted FAAs, including a *N*′-methylamide, *N*′-benzhydrylamide, and *N*′-benzylamide, and found that the *N*′-benzylamide unit is necessary for the excellent anticonvulsant activity of FAAs.[15] We substituted the aromatic ring in (*R*)-**3** for a cyclohexyl ring ((*R*)-**18**) to examine the influence of the planar ring system on anticonvulsant activity.

α-Substituted *N*-benzylbutanamides (Site F) and *N*-Substituted C(2)-Isopropyl PAADs (Site F′)

In our final analysis we examined the importance of the amino group and amine substitution. The limited availability of 2-substituted 3-methylbutanoic acid reagents prompted the investigation of *N*-benzylbutanamide derivatives ((*R*)-**19**, **20**, (*R,S*)-**21**, (*S*)-**21**, and (*R*)-**22**) instead of *N*′-benzyl 3-methylbutanamide derivatives (Site F). Previously, the C(2)-acetamido of (*R,S*)-*N*′-benzyl 2-acetamido-2-phenylacetamide ((*R,S*)-**25**, ED_{50} = 20 mg/kg) was systematically replaced with a hydrogen, methyl, hydroxy, methoxy, or halogen group. The hydroxy and methoxy groups provided moderate MES activities (ED_{50} = >30, <100 mg/kg) but were appreciably less active than the parent FAA.[16] In a similar study, the C(2)-acetamido group of (*R,S*)-*N*′-benzyl 2-acetamido-3-methoxypropionamide ((*R,S*)-**26**, ED_{50} = 8.3 mg/kg) was replaced with a methyl, hydroxy, and amino group. The methyl and amino groups also resulted in moderate MES activities (ED_{50} = >30, <100 mg/kg) but, again, the activities were lower than the corresponding FAA.[17] Accordingly, both studies concluded that the C(2)-acetamido group showed superior anticonvulsant activity. We conducted a similar investigation in which we replaced the amino group in (*R*)-**19** with a hydrogen (**20**), methyl ((*R,S*)-**21**), or hydroxy group ((*R*)-**22**). The moderate activity of (*R,S*)-**21** prompted our efforts to prepare (*R*)-**21** and (*S*)-**21** to determine if anticonvulsant activity resided in the (*R*)-stereoisomer. We successfully synthesized (*S*)-**21** but were unable to prepare (*R*)-**21**.

Finally, to assess the importance of *N*-terminal substitution (Site F′), we prepared the Secondary Amino Acid Derivative (SAAD) (*R*)-**23** and the Tertiary Amino Acid Derivative (TAAD) (*R*)-**24** in the C(2)-isopropyl series. Earlier studies did not reveal a general pattern to correlate anticonvulsant activity with amine substitution.[18–20] We observed that *N*-amine substitution improved anticonvulsant activity in several cases, but in other compounds, we saw a loss of activity.



(*R,S*)-**25** R = C_6H_5

(*R,S*)-**26** R = CH_2OCH_3

Chemistry

Thioamide (*R*)-**7** was prepared by treating (*R*)-**27**[6] with excess Lawesson's reagent at reflux,[12] followed by trifluoroacetic acid (TFA) deprotection of the *t*-Boc group (Scheme 1). We were mindful of the potential for thiation of the carbamate carbonyl in (*R*)-**27,** but the ^{13}C NMR spectrum contained a signal at ~155 ppm, the typical shift for a carbamate carbon, rather than ~190 ppm for the thiocarbamate.[21] In agreement with the proposed structure of (*R*)-**27**, we observed the thioamide carbon signal in the ^{13}C NMR at 205 ppm.

Diamine (*R*)-**8**[14] was synthesized by directly reducing (*R*)-**3**[6] with borane in THF (Scheme 2). We followed a previously reported procedure[14] but changed the workup.

We synthesized PAAD (*R*)-**9** by coupling (*R*)-**29**[6] with *N*,*O*-dimethylhydroxylamine hydrochloride (HCl) in the presence of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CMDT) and base[22] to give Weinreb amide (*R*)-**30** (Scheme 3). Weinreb amides readily react with Grignard reagents and are widely known as useful precursors to ketones.[22–24] Accordingly, (*R*)-**30** was reacted with phenethylmagnesium bromide to give (*R*)-**31**, followed by HCl deprotection to give PAAD (*R*)-**9** as a HCl salt.

We synthesized (*R*)-**32** from (*R*)-**29**[6] using a mild esterification method [25] that employed benzylchloroformate in the presence of base and catalytic amounts of 4-dimethylaminopyridine (DMAP) (Scheme 4). Subsequent TFA deprotection of (*R*)-**32** gave PAAD (*R*)-**10**.

The C(2)-isopropyl PAAD analogs (*R*)-**11**–**16** and (*R*)-**18** were synthesized by coupling (*R*)-**29**[6] with commercially available amines (**33**–**39**) and using the standard mixed anhydride coupling (MAC) procedure,[26] to give amides (*R*)-**40**–**46**, followed by TFA deprotection to the corresponding PAADs (Scheme 5).

PAADs **20,** (*R*,*S*)-**21**, (*S*)-**21**, and (*R*)-**22** were synthesized from commercially available carboxylic acids **47**, (*R*,*S*)-**48**, (*S*)-**48**, and (*R*)-**49** (Scheme 6). For (*R*)-**20**, (*R*,*S*)-**21**, and (*S*)-**21**, we used the MAC procedure with benzylamine. We attempted to prepare (*R*)-**21** to compare its pharmacological activity with (*S*)-**21** but were unsuccessful.[27] PAAD (*R*)-**22** was prepared by treating (*R*)-**49** with benzylamine in the presence of the commercially available catalyst, 3,5- bis(trifluoromethyl)benzeneboronic acid[28] (**50**) (Scheme 6).

SAAD (*R*)-**23** was prepared in three steps, beginning with the *t*-Boc protection of commercially available acid (*R*)-**51** to give (*R*)-**52** (Scheme 7). (*R*)-**52** was coupled with benzylamine using the MAC procedure to give amide (*R*)-**53** and then HCl deprotected to give SAAD (*R*)-**23** as the HCl salt.

Synthesis of TAAD (*R*)-**24** began with reductive condensation of D-valine ((*R*)-**54**) with formaldehyde using 10% Pd-C in the presence of H_2 to give (*R*)-**55** (Scheme 8).[29–31] (*R*)-**55** was coupled with benzylamine using the condensing reagent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4- methylmorpholinium chloride (DMTMM)[32] to give TAAD (*R*)-**24**.

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 compounds prepared and their physical and spectroscopic properties.

Pharmacological Activity

PAADs **7**–**22** and **24** were evaluated for anticonvulsant activity using the MES test at the National Institute of Neurological Disorders and Stroke Anticonvulsant Screening Program

(NINDS ASP), following the procedures described by Stables and Kupferberg,[5] and described in the preceeding paper.[8] PAAD **23** was evaluated for anticonvulsant activity at UCB Pharma, following the procedures described by Klitgaard,[33] as previously reported.[8] The pharmacological data from the MES tests are summarized in Tables 1 and 2. PAADs tested at the NINDS ASP were evaluated in the subcutaneous Metrazol® (scMet) seizure model[5] and displayed little to no protection.[27]

Compound (*R*)-**3** emerged as a potent anticonvulsant (MES ED_{50} = 15 mg/kg) that possessed pain attenuating properties (formalin ED_{50} = 15 mg/kg) from the SAR investigation at the C(2)-carbon.[6] This excellent activity came as a surprise since it did not follow the trends observed in the FAA series. Therefore, we have questioned several aspects of the original PAAD structural framework to determine if the basic tenets of the FAA blueprint applied to this C(2)- hydrocarbon PAAD. We investigated the importance of six properties (Sites A–F) that were common in the FAAs and report their anticonvulsant activity and neurological toxicity in Tables 1 and 2.

First, we determined the effect of the carbonyl unit (Site A) on anticonvulsant activity (Table 1). Replacement of the carbonyl unit in (*R*)-**3** with a thiocarbonyl unit to give (*R*)-**7** resulted in decreased MES activity in mice (ED_{50} (mg/kg): (*R*)-**3**, 15; (*R*)-**7**, >30, <100), but notable activity was observed in rats (ED_{50} = <30 mg/kg). Reducing the carbonyl unit in (*R*)-**3** to a methylene group to provide (*R*)-**8** led to decreased activity in both mice (ED_{50} = >30, <100 mg/kg) and rats (ED_{50} = >30 mg/kg).

Next, we determined the effect of the amide bond (Site B) on anticonvulsant activity (Table 1). Converting the amide (*R*)-**3** (Y = NH) to ketone (*R*)-**9** (Y = CH_2) decreased the MES activity (ED_{50} (mg/kg): (*R*)-**3**, 15; (*R*)-**9**, >30, <100) and conversion to the ester (*R*)-**10** (Y = O) abolished activity (ED_{50} = >300 mg/kg).

Then, at Site C we looked at the optimal methylene linker length between the amide bond and the aromatic ring (Table 1). Direct linkage of the aromatic ring to the amide bond to give (*R*)-**11** (n = 0) resulted in a significant drop in activity from the parent compound (*R*)-**3** (n = 1) (ED_{50} (mg/kg): (*R*)-**3**, 15; (*R*)-**11**, >30, <100). However, extending the linkage of the parent compound (*R*)-**3** (n = 1) by one methylene unit to provide (*R*)-**12** (n = 2) increased anticonvulsant activity by ~30% (ED_{50} = 10 mg/kg). The activity increase was associated with a neurotoxicity increase over the parent compound (TD_{50} (mg/kg): (*R*)-**3**, 70; (*R*)-**12**, 50); however, the PI of (*R*)-**3** (4.8) and (*R*)-**12** (5.0) were nearly equal. Extending the linker by another carbon to give (*R*)-**13** (n = 3) led to comparable activity with (*R*)-**3** (ED_{50} (mg/kg): (*R*)-**3**, 15; (*R*)-**13**, 16), but the increased toxicity for (*R*)-**13** (TD_{50} = 43 mg/kg) reduced the PI to 2.7. A final extension to n = 4 to give (*R*)-**14** resulted in comparable activity in mice (ip) with (*R*)-**3** and (*R*)- **13** (ED_{50} (mg/kg): (*R*)-**14**, 11; (*R*)-**3**, 15; (*R*)-**13**, 16), with a PI of 3.5. The anticonvulsant activities of (*R*)-**12**–**14** were surprising since in the FAA series, an increase in the methylene linkage led to a sharp drop in activity.[34]

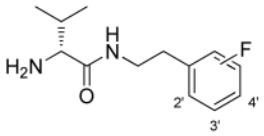
Site D compared the effect of substitution on the *N*′-benzylamide ring, where we systematically placed a trifluoromethoxy group at the 2′ ((*R*)-**15**), 3′ ((*R*)-**16**), and 4′-positions ((*R*)-**17**) (Table 1). All regiosubstitutions displayed excellent MES activities in mice (ED_{50} (mg/kg): (*R*)-**15**, 9.2; (*R*)-**16**, 7.1; (*R*)-**17**, 16). The ED_{50} value for (*R*)-**16** was 2-fold more active than the parent compound (*R*)-**3** (ED_{50} = 15 mg/kg) but the increase in activity was associated with an increase in neurotoxicity (ED_{50} (mg/kg): (*R*)-**3**, 70; (*R*)-**16**, 30). Comparison of the MES activity in rats (Table 1) showed ~3-fold drop in activity going from (*R*)-**3** to (*R*)-**15** (ED_{50} (mg/kg): (*R*)-**3**, 11; (*R*)-**15**, 33). The MES activity of (*R*)-**16** in the rat (ED_{50} = 10 mg/kg) was comparable with (*R*)-**3** (ED_{50} = 11 mg/kg) but there was a sharp increase in behavioral toxicity (TD_{50} (mg/kg): (*R*)-**3**, >500; (*R*)-**16**, 43). The potent

anticonvulsant activity observed for (*R*)-**15** led us to evaluate this compound in the formalin neuropathic pain model[35] where we observed excellent activity in the inflammatory phase (ED_{50} = 9.2 mg/kg, data not shown).[36]

Next, we examined the need for a benzyl moiety (Site E) (Table 1). We replaced the aromatic ring ((*R*)-**3**) with a cyclohexyl ring ((*R*)-**18**) and observed a decrease in anticonvulsant activity (ED_{50} (mg/kg): (*R*)-**3**, 15; (*R*)-**18**, >30, <100). When (*R*)-**18** was evaluated in the rat, we found this PAAD to have excellent activity (ED_{50} = ~15 mg/kg). While our data indicates that the *N*′-benzyl substituent is preferred over the saturated *N*′-cyclohexylmethyl unit, we did not anticipate the anticonvulsant activity of (*R*)-**18**.

Finally, we investigated the importance of the C(2)-amino functionality (Site F) by comparing the unsubstituted *N*-benzyl butanamide **20** (X = H), the methyl-substituted *N*-benzyl butanamide (*R*,*S*)-**21** and (*S*)-**21** (X = CH_3), and the hydroxy-substituted *N*-benzyl butanamide (*R*)-**22** (X = OH) with the parent PAAD (*R*)-**19** (X = NH_2) (Table 2). Compounds **20** and (*R*)-**22** gave similar, modest activity (ED_{50} = >30, <100 mg/kg) in mice and no appreciable activity in rats (ED_{50} = >30 mg/kg). (*R*,*S*)-**21** also displayed modest protection in mice (ED_{50} = 56 mg/kg) with minimal neurotoxicity (TD_{50} = 165 mg/kg), and moderate activity in rats (ED_{50} = 51 mg/kg) without any detectable behavioral toxicity (TD_{50} = >500 mg/kg). We were surprised by the activity of (*R*,*S*)-**21** since the methyl group lacks the hydrogen bonding capabilities afforded by an amino or a hydroxy group. Evaluation of (*S*)-**21** showed a decrease in anticonvulsant activity (ED_{50} = >100, <300 mg/kg), suggesting that activity resided predominantly in the (*R*)-isomer. Unfortunately, we were unable to complete the synthesis of (*R*)-**21** to confirm this suggestion. The activities of the primary (PAAD), secondary (SAAD), and tertiary (TAAD) amino acid analogs of (*R*)-*N*′-benzyl 2-amino-3-methylbutanamide ((*R*)-**3**) are summarized in Table 2 (Site F′). Comparison of the SAAD (*R*)-**23** with the PAAD (*R*)-**3** showed decreased activity in the MES test (ED_{50} (mg/kg): (*R*)-**3**, 15; (*R*)-**23**, 25). Furthermore, comparison of the TAAD (*R*)-**24** with the SAAD (*R*)-**23** showed an additional decrease in activity in the MES test (ED_{50} (mg/kg): (*R*)-**23**, 25; (*R*)-**24**, >30, <100). Therefore, the MES data for PAAD (*R*)-**3**, SAAD (*R*)-**23**, and TAAD (*R*)-**24** revealed a linear decrease in anticonvulsant activity as we successively incorporated methyl groups at the C(2)-amine site. This trend differed from the reported pattern for racemic C(2)-methyl and C(2)-CH_2OCH_3 PAADs, in which successive *N*-methylation led to non-linear trends in MES activity.[18]

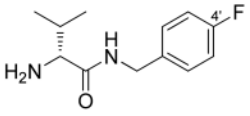
Our findings indicated that PAAD anticonvulsant activity increased when a *N*-phenethylamide moiety (Table 1, (*R*)-**12**) was used and when an electron-withdrawing substituent (OCF_3) was incorporated on the aromatic ring (Table 1, (*R*)-**15**–**17**). Accordingly, we prepared three mono-fluoro regioisomers of (*R*)-*N*-phenethyl 2-amino-3-methylbutanamide, ((*R*)- **56**–**58**) using the same general procedure outlined in Scheme 5. We observed that anticonvulsant activity remained high in the MES test (mice, ip) for all three regioisomers (ED_{50} (mg/kg): (*R*)- **56**, 27; (*R*)-**57**, 20; (*R*)-**58**, >10, <30), but there was no apparent benefit over the parent, unsubstituted PAAD (*R*)-**12** (MES ED_{50} = 10 mg/kg). However, we observed an increase in activity and a decrease in toxicity in the MES test for (*R*)-**56** going from mice (ip) to rats (po). (*R*)-**58** exhibited only slightly better activity than the corresponding benzyl analog (*R*)-**59**[8] (ED_{50} = 32 mg/kg), in terms of potency in the MES test and PI values (Table 1).



(*R*)-**56** 2'-F

(*R*)-**57** 3'-F

(*R*)-**58** 4'-F



(*R*)-**59**

CONCLUSIONS

Examination of PAAD Sites A–F revealed that the amide bond (Sites A and B) was important for effective seizure protection and altering this unit affected the hydrogen bonding properties of the compounds. Assessment of the methylene linker (Site C) showed that at least one carbon between the amide bond and the aromatic rings was necessary for anticonvulsant activity but incorporation of up to three additional methylene units provided excellent activity. When considering activity, toxicity, and the protective index for these C(2)-isopropyl PAADs, the optimal linker length appeared to be when n = 2, instead the n = 1, as seen in the FAAs. Similarly, we found that replacement of the benzylamide group by a cyclohexylmethylamide (Site E) led to lower anticonvulsant activity, but the drop was modest. Thus, there seems to be structural latitude at Sites C and E, where modifications retain excellent activities. Comparison of trifluoromethoxy regioisomers of (*R*)-*N*′-benzyl 2-amino-3-methylbutanamide (Site D) revealed that superb seizure protection was associated with all positions. The activity increase was associated with a neurotoxicity increase but the PI values were comparable with the parent, unsubstituted PAAD (*R*)-**3**. Lastly, we evaluated our choice of the C(2)-amino group (Site F) since the earlier SAR studies were centered around primary amino acid derivatives.[6, 8] Of the C(2)-groups investigated, the amino group was most active but was not mandatory for activity. The activities of the primary (PAAD), secondary (SAAD), and tertiary (TAAD) amino acid analogs of (*R*)-*N*′-benzyl 2-amino-3-methylbutanamide ((*R*)-**3**) showed a steady loss of anticonvulsant activity with *N*-methylation. Collectively, the findings of this study, along with the earlier observations that the anticonvulsant activity of C(2)-hydrocarbon PAADs do not improve when a substituted heteroatom is included one atom removed from the C(2)-center[6] and that PAAD activity is sensitive to the electronic effects of *N*′-benzylamide substituents,[8] indicated that the C(2)-hydrocarbon PAADs have an unique SAR and. Future work will investigate PAAD mechanism of action to determine if the difference in observed efficacy of PAADs and FAAs is a consequence of varying pharmacokinetics or if PAADs interact with a novel molecular target.

EXPERIMENTAL SECTION

General methods

The general methods used in this study were identical to those previously reported[6] and are summarized in the Supporting Information. All compounds were checked by TLC, ^{1}H 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 (3x). The combined aqueous layers were washed with CH_2Cl_2 (2x), basified (pH 10–12) with aqueous 4 M NaOH, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine (2x), 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 (3x). The aqueous layers were combined and washed with CH_2Cl_2 (2x). All of the CH_2Cl_2 layers were combined and successively washed with H_2O (2x) and brine (2x), dried (Na_2SO_4), evaporated in vacuo, and purified by column chromatography (SiO_2).

General Procedure for the Preparation of *N′*-Benzylamide Amino Acid Derivatives Using the Mixed Anhydride Coupling (MAC) Method (Method B)

An anhydrous THF solution of carboxylic acid (0.5–2.0 M) was cooled to −78 °C in a dry ice/acetone bath under an inert atmosphere (Ar or N_2), and 4-methylmorpholine (NMM) (1.3–1.5 equiv) was added. After the mixture was stirred (2–10 min), isobutyl chloroformate (IBCF) (1.1–1.5 equiv) was added leading to the precipitation of a white solid. The reaction was allowed to proceed for an additional 15–25 min, and then benzylamine (1.05–1.36 equiv) was added at −78 °C. The reaction mixture was allowed to stir at room temperature (1.5 h), and then the insoluble salts were filtered. The organic layer was concentrated in vacuo, and the product was purified by column chromatography (SiO_2).

(*R*)-*N′*-Benzyl 2-Amino-3-methylbutanethioamide ((*R*)-7)

Utilizing Method A with (*R*)-**28** (1.72 g, 5.34 mmol), TFA (5.95 mL, 80.1 mmol), and CH_2Cl_2 (18 mL) gave the crude product after acidic workup and 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 (0.89 g, 76%) as a yellow oil: $[\alpha]^{28.5}_D$ +43.7° (*c* 1.0, $CHCl_3$); R_f 0.50 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (d, *J* = 6.8 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 3H), 1.36 (s, 2H), 2.75–2.87 (m, 1H), 3.78 (d, *J* = 2.8 Hz, 1H), 4.84 (dd, *J* = 4.6, 15.0 Hz, 1H), 4.91 (dd, *J* = 5.0, 15.0 Hz, 1H), 7.29–7.38 (m, 5H), 9.75–9.95 (br s, 1H); LRMS (ESI) 223.13 [M + H^+] (calcd for $C_{12}H_{18}N_2SH^+$ 223.14); Anal. ($C_{12}H_{18}N_2S$): C, H, N.

(*R*)-1-*N′*-Benzylamino-2-amino-3-methylbutane ((*R*)-8).[37]

(*R*)-**3**[6] (3.38 g, 16.4 mmol) was dissolved in anhydrous THF (65 mL) and cooled to 0 °C. BH_3•THF (1 M, 49.19 mL, 49.19 mmol) was added dropwise and the mixture was heated to reflux (18 h). The mixture was cooled to 0 °C and aqueous 0.5 M HCl (100 mL) was slowly added. The THF was evaporated in vacuo and Et_2O (100 mL) was added to the acidic aqueous solution. The aqueous layer was separated and the organic layer was extracted with aqueous 0.5 M HCl (3 × 50 mL). All of the aqueous layers were combined and washed with Et_2O (2 × 100 mL). The aqueous layer was basified to pH 10–12 using aqueous 4 M NaOH and extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 layers were combined and were successively washed with a 1:1 mixture of EtOH/H_2O (2 × 100 mL) and saturated aqueous brine (2 × 100 mL), dried (Na_2SO_4), evaporated in vacuo, and purified three times by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2) to give the desired product (0.53 g, 17%) as a pale yellow oil: $[\alpha]^{28}_D$ −31.4° (*c* 0.51, CH_2Cl_2) (lit.[37] $[\alpha]^{20}_D$ − 30.4° (*c* 0.55, CH_2Cl_2)), $[\alpha]^{28}_D$ −31.4° (*c* 1.9, $CHCl_3$) (lit.[38] (*S*): $[\alpha]^{25.4}_D$ +33.4° (*c* 1.80, $CHCl_3$)); R_f 0.53 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, *J* = 6.8 Hz, 6H), 1.58–1.69 (m, 1H), 2.42–2.48 (m, 4H), 2.64–2.69 (m, 1H), 2.74 (dd, *J* = 3.2, 12.0 Hz, 1H), 3.78 (1/2 AB_q, *J* = 13.4 Hz, 1H), 3.84 (1/2 AB_q, *J* = 13.4 Hz, 1H), 7.22–7.35 (m, 5H); HRMS (ESI) 193.1705 [M + H^+] (calcd for $C_{12}H_{20}N_2H^+$ 193.1695); Anal. ($C_{12}H_{20}N_2$•H_2O): C, H, N.

(*R*)-4-Amino-*2*-methyl-6-phenyl-4-hexanone Hydrochloride ((*R*)-9)

(*R*)-**31**[39, 40] (3.04 g, 9.96 mmol) was dissolved in MeOH (50 mL) and aqueous concentrated HCl (4.9 mL, 59 mmol) was added. The reaction was heated at reflux (1 h) and then cooled to room temperature before evaporating the solvent in vacuo to give a crude oil. The crude product was triturated with hexanes (3x) to give the desired product (0.60 g, 25%) as a white solid: mp 123–124 °C; $[\alpha]^{28}_{D}$ − 59.7° (*c* 1.1, $CHCl_3$); R_f 0.49 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (d, *J* = 7.0 Hz, 3H), 1.17 (d, *J* = 7.0 Hz, 3H), 2.34–2.44 (br m, 1H), 2.56–2.98 (m, 4H), 4.18–4.26 (br d, 1H), 7.16–7.24 (m, 5H), 8.57 (br s, 3H); LRMS (ESI) 206.13 [M – Cl^-] (calcd for $C_{13}H_{20}NO$ 206.13); Anal. ($C_{13}H_{20}ClNO$): C, H, Cl, N.

(*R*)-*O*-Benzyl 2-Amino-3-methylbutanoate ((*R*)-10).[41]

1 M HCl in Et_2O (165 mL) was added to a Et_2O solution (10 mL) of (*R*)-**32**[25, 42] (2.02 g, 6.58 mmol) at 0 °C. The reaction was stirred at room temperature (18 h) to give a cloudy white solution. The solution was filtered but no substantial filtrate was collected. The solvent was evaporated in vacuo to give a crude oil that was then redissolved in CH_2Cl_2 (10 mL). The organic layer was extracted with aqueous 1 M HCl (3 × 10 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 × 30 mL). The aqueous layer was basified to pH 10–12 with aqueous 4 M NaOH and extracted with CH_2Cl_2 (3 × 50 mL). The second set of organic layers were combined and washed with saturated aqueous brine (2 × 150 mL), dried (Na_2SO_4), and evaporated in vacuo. The crude product was purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (0.99 g, 72%) as a pale yellow oil: $[\alpha]^{28.5}_{D}$ −10.2° (*c* 1.1, $CHCl_3$); R_f 0.39 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 1.44 (s, 2H), 1.99–2.10 (m, 1H), 3.34 (d, *J* = Hz, 1H), 5.14 (1/2 AB_q, *J* = 12.2 Hz, 1H), 5.18 (1/2 AB_q, *J* = 12.2 Hz, 1H), 7.30–7.39 (m, 5H); LRMS (ESI) 208.15 [M + H^+] (calcd for $C_{12}H_{17}NO_2H^+$ 208.15); Anal. ($C_{12}H_{17}NO_2$): C, H, N.

(*R*)-*N′*-Phenyl 2-Amino-3-methylbutanamide ((*R*)-11).[43]

Utilizing Method A with (*R*)-**40**[44] (1.75 g, 5.99 mmol), TFA (6.67 mL, 89.8 mmol), and CH_2Cl_2 (20 mL) gave the crude product after acidic workup and 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 (0.94 g, 82%) as a pale orange oil: $[\alpha]^{25}_{D}$ +82.5° (*c* 1.6, CH_2Cl_2); R_f 0.34 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (d, *J* = 7.2 Hz, 3H), 1.04 (d, *J* = 7.2 Hz, 3H), 1.46 (s, 2H), 2.40–2.48 (m, 1H), 3.37 (d, *J* = 3.6 Hz, 1H), 7.09 (t, *J* = 7.2 Hz, 1H), 7.32 (t, *J* = 8.2 Hz, 2H), 7.60 (d, *J* = 8.2 Hz, 2H), 9.46–9.56 (br s, 1H); HRMS (ESI) 193.1343 [M + H^+] (calcd for $C_{11}H_{16}N_2OH^+$ 193.1341); Anal. ($C_{11}H_{16}N_2O$•$0.01H_2O$): C, H, N.

(*R*)-*N′*-Phenethyl 2-Amino-3-methylbutanamide ((*R*)-12)

Utilizing Method A with (*R*)-**41** (1.37 g, 4.28 mmol), TFA (4.77 mL, 64.2 mmol), and CH_2Cl_2 (15 mL) gave the crude product after acidic workup and 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 (873 mg, 93%) as a pale yellow solid: mp 36–37 °C; $[\alpha]^{25}_{D}$ +32.9° (*c* 1.0, CH_2Cl_2); R_f 0.19 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 7.2 Hz, 3H), 1.20–1.26 (s, 2H), 2.22–2.33 (m, 1H), 2.77–2.88 (m, 2H), 3.19 (d, *J* = 4.0 Hz, 1H), 3.46– 3.62 (m, 2H), 7.20–7.32 (m, 5H); HRMS (ESI) 221.1643 [M + H^+] (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654); Anal. ($C_{13}H_{20}N_2O$): C, H, N.

(*R*)-*N′*-Phenylpropyl 2-Amino-3-methylbutanamide ((*R*)-13)

Utilizing Method A with (*R*)-**42** (5.00 g, 15.0 mmol), TFA (16.7 mL, 225 mmol), and CH_2Cl_2 (50 mL) gave the crude product after acidic workup and 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 (3.02 g, 86%) as a pale yellow oil: $[\alpha]^{28.5}_D$ +30.1° (*c* 1.2, $CHCl_3$); R_f 0.21 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 7.2 Hz, 3H), 1.30 (s, 2H), 1.79–1.90 (m, 2H), 2.23–2.34 (m, 1H), 2.65 (t, *J* = 8.4 Hz, 2H), 3.19 (d, *J* = 4.0 Hz, 1H), 3.22–3.37 (m, 2H), 7.16–7.20 (m, 3H), 7.26–7.35 (m, 3H); HRMS (ESI) 235.1818 [M + H$^+$] (calcd for $C_{14}H_{22}N_2OH^+$ 235.1810); Anal. ($C_{14}H_{22}N_2O$•0.16H_2O): C, H, N.

(*R*)-*N*′-Phenylbutyl 2-Amino-3-methylbutanamide ((*R*)-14)

Utilizing Method A with (*R*)-**43** (3.18 g, 9.13 mmol), TFA (10.2 mL, 137 mmol), and CH_2Cl_2 (30 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 compound (2.16 g, 96%) as a pale yellow oil: $[\alpha]^{28.5}_D$ +29.9° (*c* 1.0, $CHCl_3$); R_f 0.53 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 1.51–1.59 (m, 4H), 1.62–1.70 (m, 2H), 2.22–2.34 (m, 1H), 2.63 (t, *J* = 8.0 Hz, 2H), 3.20 (d, *J* = 4.0 Hz, 1H), 3.22–3.44 (m, 2H), 7.15–7.19 (m, 3H), 7.25–7.36 (m, 3H); HRMS (ESI) 249.1954 [M + H$^+$] (calcd for $C_{15}H_{24}N_2OH^+$ 249.1967); Anal. ($C_{15}H_{24}N_2O$•0.0.6CH_2Cl_2): C, H, N.

(*R*)-2′-*N*′-(Trifluoromethoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-15)

Utilizing Method A with (*R*)-**44** (6.00 g, 15.4 mmol), TFA (17.1 mL, 231 mmol), and CH_2Cl_2 (50 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 (3.70 g, 83%) as a pale yellow solid: mp 54–55 °C; $[\alpha]^{28.5}_D$ +28.0° (*c* 1.0, $CHCl_3$); R_f 0.59 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 1.34 (s, 2H), 2.28–2.39 (m, 1H), 3.27 (d, *J* = 4.0 Hz, 1H), 4.48–4.57 (m, 2H), 7.22–7.33 (m, 3H), 7.41–7.43 (m, 1H), 7.71–7.79 (br t, 1H); LRMS (ESI) 291.12 [M + H$^+$] (calcd for $C_{13}H_{17}F_3N_2O_2H^+$ 291.12); Anal. ($C_{13}H_{17}F_3N_2O_2$): C, H, F, N.

(*R*)-3′-*N*′-(Trifluoromethoxy)benzyl 2-Amino-3-methylbutanamide ((*R*)-16)

Utilizing Method A with (*R*)-**45** (3.00 g, 7.69 mmol), TFA (8.57 mL, 115 mmol), and CH_2Cl_2 (25 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 (1.81 g, 81%) as a pale yellow oil: $[\alpha]^{28.5}_D$ +20.6° (*c* 1.3, $CHCl_3$); R_f 0.47 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 1.38 (s, 2H), 2.31–2.40 (m, 1H), 3.30 (d, *J* = 4.0 Hz, 1H), 4.43 (dd, *J* = 6.2, 15.0 Hz, 1H), 4.51 (dd, *J* = 6.6, 15.0 Hz, 1H), 7.10–7.13 (m, 2H), 7.21–7.22 (m, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 7.78–7.86 (br t, 1H); LRMS (ESI) 291.11 [M + H$^+$] (calcd for $C_{13}H_{17}F_3N_2O_2H^+$ 291.11); Anal. ($C_{13}H_{17}F_3N_2O_4$): C, H, F, N.

(*R*)-*N*′-Cyclohexylmethyl 2-Amino-3-methylbutanamide ((*R*)-18)

Utilizing Method A with (*R*)-**46** (3.60 g, 11.5 mmol), TFA (12.8 mL, 173 mmol), and CH_2Cl_2 (38 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 (2.34 g, 96%) as a white solid: mp 85–86 °C; $[\alpha]^{25}_D$ +38.1° (*c* 1.1, $CHCl_3$); R_f 0.59 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, *J* = 7.0 Hz, 3H), 0.89–0.99 (m, 2H), 0.99 (d, *J* = 7.0 Hz, 3H), 1.10–1.28 (m, 3H), 1.36 (s, 2H), 1.41–1.52 (m, 1H), 1.65–1.74 (m, 5H), 2.26–2.37 (m, 1H), 3.03–3.18 (m, 2H), 3.23 (d, *J* = 3.6 Hz, 1H), 7.30–7.40 (br t, 1H); LRMS (ESI) 213.18 [M + H$^+$] (calcd for $C_{12}H_{24}N_2OH^+$ 213.18); Anal. ($C_{12}H_{24}N_2O$): C, H, N.

N-Benzyl Butanamide (20).[45]

Utilizing Method B with **47** (2.00 mL, 21.9 mmol), NMM (3.13 mL, 28.5 mmol), IBCF (3.10 mL, 24.1 mmol), and benzylamine (2.51 mL, 23.0 mmol) gave the crude product that was recrystallized from hot EtOAc/hexanes to give the desired compound (1.18 g, 30%) as a white solid: mp 54–55 °C (lit.[45] mp 36.9–38 °C); R_f 0.67 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, J = 7.6 Hz, 3H), 1.65–1.74 (m, 2H), 2.20 (t, J = 8.0 Hz, 2H), 4.45 (d, J = 5.6 Hz, 2H), 5.66–5.78 (br s, 1H), 7.26–7.35 (m, 5H); HRMS (ESI) 178.1238 [M + H^+] (calcd for $C_{11}H_{15}NOH^+$ 178.1232); Anal. ($C_{11}H_{15}NO$•0.06H_2O): C, H, N.

(*R*,*S*)-*N*-Benzyl 2-Methylbutanamide ((*R*,*S*)-21).[46]

Utilizing Method B with (*R*,*S*)-**48** (2.00 mL, 18.3 mmol), NMM (2.62 mL, 23.8 mmol), IBCF (2.60 mL, 20.2 mmol), and benzylamine (2.10 mL, 19.3 mmol) gave the crude product that was purified twice by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes) to give the desired compound (2.32 g, 66%) as a white solid: mp 54–55 °C (lit.[46] mp 47.5–48.5 °C); R_f 0.80 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, J = 7.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.39–1.50 (m, 1H), 1.64–1.75 (m, 1H), 2.09– 2.17 (m, 1H), 4.39–4.49 (m, 2H), 5.79–5.89 (br s, 1H), 7.26–7.35 (m, 5H); HRMS (ESI) 214.1199 [M + Na^+] (calcd for $C_{12}H_{17}NOH^+$ 214.1208); Anal. ($C_{12}H_{17}NO$): C, H, N.

(*S*)-*N*-Benzyl 2-Methylbutanamide ((*S*)-21).[47]

Utilizing Method B with (*S*)-**48** (0.90 mL, 8.27 mmol), NMM (1.18 mL, 10.8 mmol), IBCF (1.17 mL, 9.10 mmol), and benzylamine (0.95 mL, 8.6 mmol) gave the crude product that was purified twice by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes) to give the desired compound (1.01 g, 64%) as a white solid: mp 55–56 °C; $[\alpha]^{28}_D$ +15.5° (*c* 1.0, acetone) (lit.[47] $[\alpha]_D$ +16.96° (*c* 1.0, acetone)); R_f 0.80 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, J = 7.6 Hz, 3H), 1.16 (d, J = 7.6 Hz, 3H), 1.40–1.50 (m, 1H), 1.65–1.75 (m, 1H), 2.09–2.18 (m, 1H), 4.39–4.49 (m, 2H), 5.80–5.88 (br s, 1H), 7.25–7.35 (m, 5H); LRMS (ESI) 193.16 [M + H^+] (calcd for $C_{12}H_{17}NOH^+$ 193.16); Anal. ($C_{12}H_{17}NO$): C, H, N.

(*R*)-*N*-Benzyl 2-Hydroxybutanamide ((*R*)-22)

To anhydrous toluene (80 mL) was added (*R*)- **49** (1.70 g, 16.3 mmol), benzylamine (1.78 mL, 16.3 mmol), and 3,5-bis(trifluoromethyl)benzene boronic acid (**50**) (0.42 g, 1.6 mmol). A pressure equalizing dropping funnel containing a cotton plug was filled 1/3 of the way with 3 Å molecular sieves that were oven dried (120 °C) and a condenser was placed above the dropping funnel. The mixture was heated at reflux (18 h) before cooling to room temperature and then the solvent was evaporated in vacuo. The crude product was purified 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 compound (1.05 g, 33%) as a white solid: mp 63–64 °C; $[\alpha]^{28.5}_D$ +28.3° (*c* 2.2, $CHCl_3$); R_f 0.56 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, J = 7.6 Hz, 3H), 1.63–1.74 (m, 1H), 1.82–1.92 (m, 1H), 3.32 (d, J = 4.8 Hz, 1H), 4.07–4.11 (m, 1H), 4.38–4.48 (m, 2H), 6.95– 7.20 (br t, 1H), 7.24–7.34 (m, 5H); LRMS (ESI) 216.12 [M + Na^+] (calcd for $C_{11}H_{15}NO_2Na^+$ 216.12); Anal. ($C_{11}H_{15}NO_2$): C, H, N.

(*R*)-*N*′-Benzyl *N*-(Methyl)amino-2-methylbutanamide Hydrochloride ((*R*)-23)

HCl in dioxane (4 mL, 4 M) was added at 0 °C to an Et_2O (1 mL) solution of (*R*)-**53** (620 mg, 4.7 mmol). The reaction was stirred at room temperature (16 h) and then concentrated in vacuo. The residue was triturated with Et_2O to give the desired compound (450 mg, 90%) as a white solid: mp 120–125 °C; $[\alpha]^{27}_D$ +93.8°(*c* 0.5, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.02 (d, J = 7.2 Hz, 3H), 1.05 (d, J = 6.9 Hz, 3H), 2.18–2.24 (m, 1H), 2.65 (s, 3H), 3.62 (d,

J = 5.1 Hz, 1H), 4.40 (1/2 AB_q, *J* = 15.0 Hz, 1H), 4.50 (1/2 AB_q, *J* = 15.0 Hz, 1H), 7.28–7.34 (m, 5H); HRMS (ESI) 221.1654 [M+H^+] (calcd. for $C_{13}H_{20}N_2OH^+$ 221.1653); Anal. ($C_{13}H_{20}N_2O$): C, H, Cl, N.

(*R*)-*N*′-Benzyl *N*,*N*-Dimethylamino-3-methylbutanamide ((*R*)-24)

(*R*)-**55** (2.28 g, 15.7 mmol) and benzylamine (2.06 mL, 18.9 mmol) were added to anhydrous THF (160 mL) at room temperature. The mixture stirred (15 min) and then DMTMM (5.22 g, 18.9 mmol) was added in one portion. The reaction continued at room temperature overnight (18 h) and then the insoluble salts were filtered, evaporated in vacuo, and purified by flash column chromatography (SiO_2; 2:1 EtOAc/CH_2Cl_2 followed by 1:10 MeOH/CH_2Cl_2) to give the desired product (0.38 g, 10%) as a white solid: mp 78–79 °C; $[\alpha]^{28}_D$ −11.2° (*c* 0.5, $CHCl_3$); R_f 0.55 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, *J* = 6.6 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 2.04–2.17 (m, 1H), 2.25 (s, 6H), 2.48 (d, *J* = 6.0 Hz, 1H), 4.42–4.51 (m, 2H), 6.58–6.65 (br t, 1H), 7.25–7.35 (m, 5H); LRMS (ESI) 235.17 [M + H^+] (calcd for $C_{14}H_{22}N_2OH^+$ 235.17); Anal. ($C_{14}H_{22}N_2O$): C, H, N.

(*R*)-2′-*N*′-(Fluoro)phenethyl 2-Amino-3-methylbutanamide ((*R*)-56)

Utilizing Method A with (*R*)-2′-*N*′-(fluoro)phenethyl 2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanamide (9.00 g, 26.6 mmol), TFA (30 mL, 0.40 mol), and CH_2Cl_2 (90 mL) gave the crude product after acidic workup and 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 (5.76 g, 91%) as a white solid: mp 51–52 °C; $[\alpha]^{28}_D$ +32.6° (*c* 1.5, $CHCl_3$); R_f 0.26 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H), 1.25 (s, 2H), 2.24–2.32 (m, 1H), 2.82–2.93 (m, 2H), 3.19 (d, *J* = 3.6 Hz, 1H), 3.46–3.61 (m, 2H), 6.99–7.09 (m, 2H), 7.17–7.23 (m, 2H), 7.34–7.42 (br t, 1H); LRMS (ESI) 239.13 [M + H^+] (calcd for $C_{13}H_{19}FN_2OH^+$ 239.13); Anal. ($C_{13}H_{19}FN_2O$): C, H, F, N.

(*R*)-3′-*N*′-(Fluoro)phenethyl 2-Amino-3-methylbutanamide ((*R*)-57)

Utilizing Method A with (*R*)-3′-*N*-(fluoro)phenethyl 2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanamide (8.50 g, 25.1 mmol), TFA (28 mL, 0.38 mol), and CH_2Cl_2 (85 mL) gave the crude product after acidic workup and 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 (5.08 g, 85%) as a pale yellow oil: $[\alpha]^{28}_D$ +31.4° (*c* 1.1, $CHCl_3$); R_f 0.26 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 1.23 (s, 2H), 2.23–2.34 (m, 1H), 2.77–2.88 (m, 2H), 3.20 (d, *J* = 4.0 Hz, 1H), 3.45–3.61 (m, 2H), 6.89–6.93 (m, 2H), 6.97–6.99 (m, 1H), 7.23–7.28 (m, 1H), 7.34–7.44 (br t, 1H); HRMS (ESI) 239.1553 [M + H^+] (calcd for $C_{13}H_{19}FN_2OH^+$ 239.1560); Anal. ($C_{13}H_{19}FN_2O$•0.01H_2O): C, H, F, N.

(*R*)-4′-*N*′-(Fluoro)phenethyl 2-Amino-3-methylbutanamide ((*R*)-58)

Utilizing Method A with (*R*)-4′-*N*′-(fluoro)phenethyl 2-*N*-(*t*-butoxycarbonyl)amino-3-methylbutanamide (9.00 g, 26.6 mmol), TFA (30 mL, 0.40 mol), and CH_2Cl_2 (90 mL) gave the crude product after acidic workup and 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 (5.82 g, 92%) as a pale yellow oil: $[\alpha]^{28}_D$ +31.8° (*c* 1.5, $CHCl_3$); R_f 0.19 (1:20 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 1.23 (s, 2H), 2.22–2.33 (m, 1H), 2.74–2.85 (m, 2H), 3.19 (d, *J* = 3.6 Hz, 1H), 3.42–3.59 (m, 2H), 6.95–7.00 (m, 2H), 7.13–7.18 (m, 2H), 7.34– 7.42 (br t, 1H); LRMS (ESI) 239.13 [M + H^+] (calcd for $C_{13}H_{19}FN_2OH^+$ 239.13); Anal. ($C_{13}H_{19}FN_2O$): 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."[48] Pharmacological evaluation by UCB Pharma consisted of the MES test[33] to assess anticonvulsant activity and the rotorod test[33] 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 $test_5$ 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).[5]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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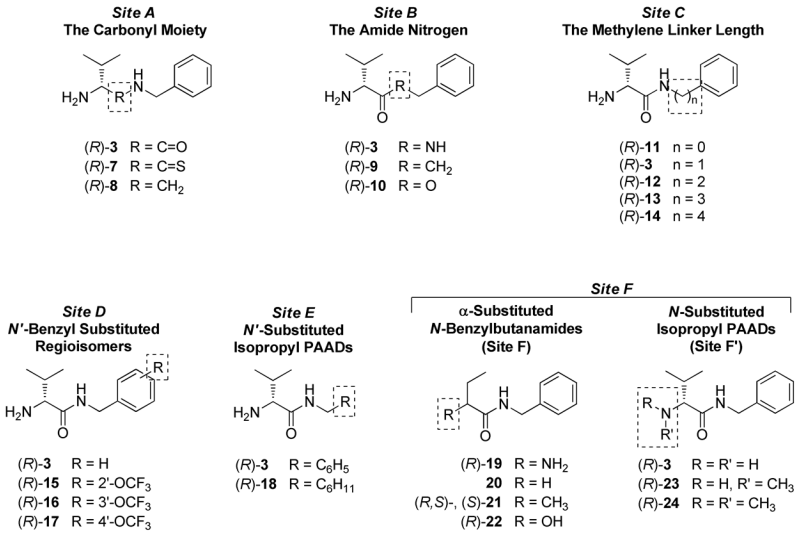
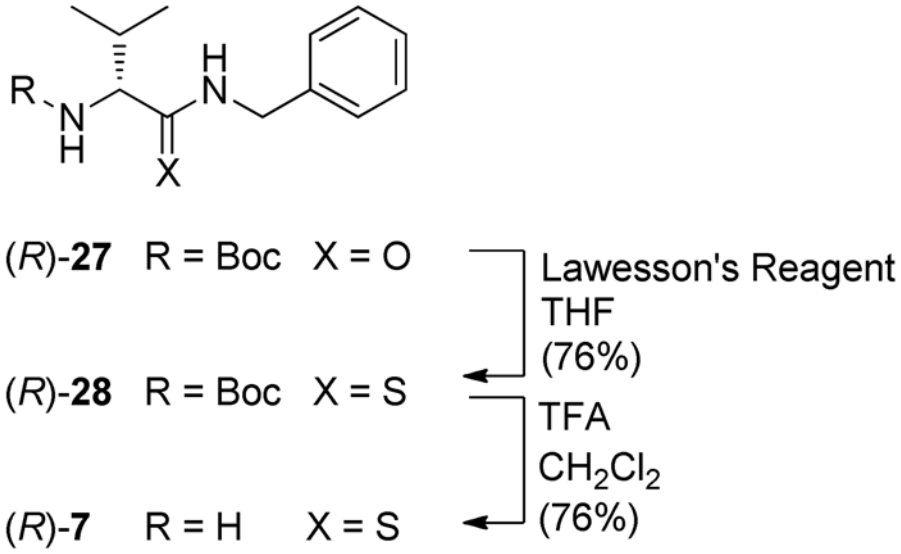
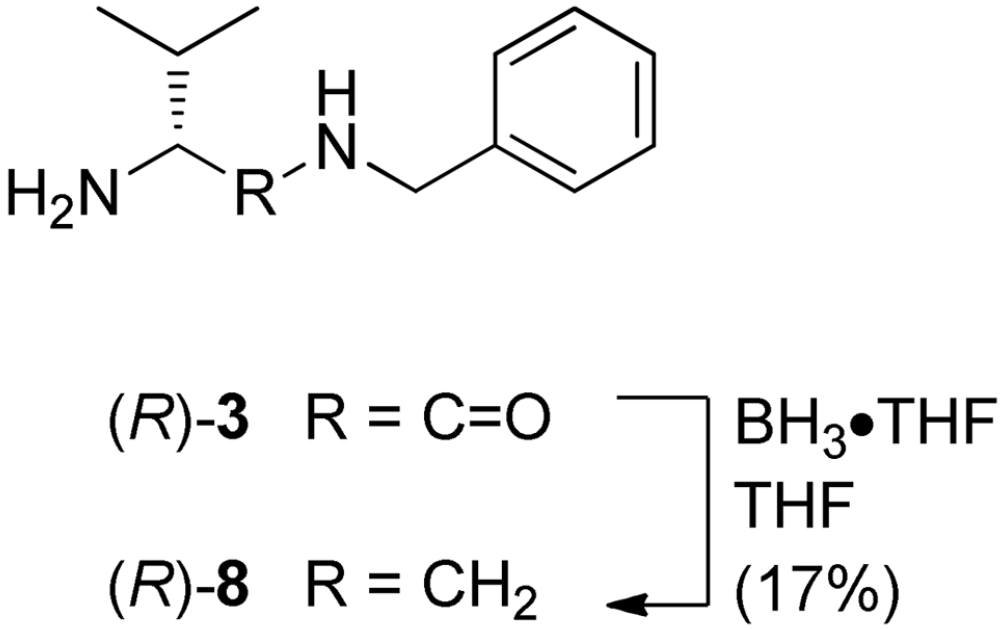


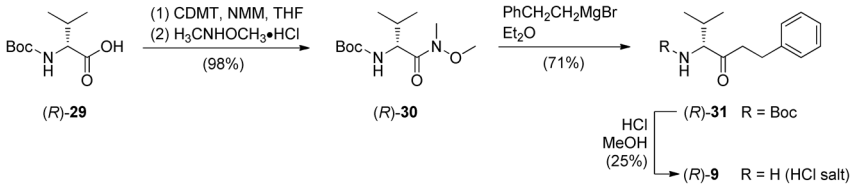
Figure 1.
C(2)-Isopropyl PAAD (*R*)-3 Analogs (Sites A-F)



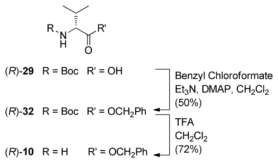
Scheme 1.
Synthesis of (*R*)-*N*′-benzyl 2-amino-3-methylthiobutanamide ((*R*)-7)



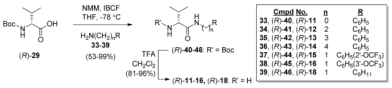
Scheme 2.
Synthesis of (*R*)-1-*N*-benzylamino-2-amino-3-methylbutane ((*R*)-8)



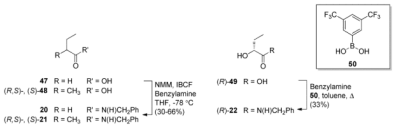
Scheme 3.
Synthesis of (*R*)-4-amino-*2*-methyl-6-phenyl-4-hexanone hydrochloride ((*R*)- 9)



Scheme 4.
Synthesis of (*R*)-*O*-benzyl 2-amino-3-methylbutanoate ((*R*)-10)

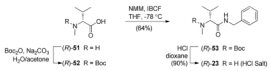


Scheme 5.
Synthesis of C(2)-isopropyl PAADs (*R*)-11–16 and (*R*)-18

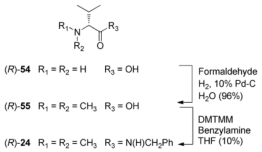


Scheme 6.

Synthesis of C(2)-isopropyl analogs: α-Substituted *N*-benzylbutanamides 20, (*R*,*S*)-21, (*S*)-21, and (*R*)-22



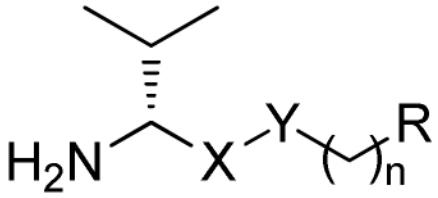
Scheme 7.
Synthesis of (*R*)-*N*′-benzyl *N*,*N*-dimethylamino-3-methylbutanamide ((*R*)-23)



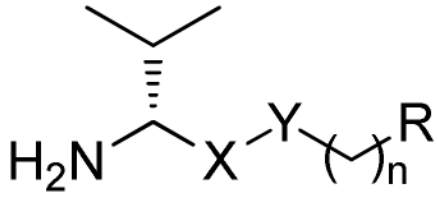
Scheme 8.
Synthesis of (*R*)-*N*′-benzyl *N*,*N*-dimethylamino-3-methylbutanamide ((*R*)-24)

Table 1

Pharmacological activities of C(2)-isopropyl PAAD amide analogs (Sites A–E) in mice (mg/kg)[a] and rats (mg/kg)[b]



						Mice (ip)[a]			Rat (po)[b]		
Cmpd No	**Site(s)**	**X**	**Y**	**n**	**R**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**3**[g]		C=O	NH	1	C_6H_5	15 [0.25] (13–18)	70 [0.25] (63–80)	4.8	11 [0.25] (9.1–13)	>500	>45
(*R*)-**7**	A	C=S	NH	1	C_6H_5	>30, <100 [0.5]	>30, <100 [0.5]		<30 [0.5–2.0]	>30 [0.25–4.0]	
(*R*)-**8**	A	CH_2	NH	1	C_6H_5	>30, <100 [0.5]	>100, <300 [0.5]		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**9**	B	C=O	CH_2	1	C_6H_5	>30, <100	>100, <300		ND[h]	ND[h]	
(*R*)-**10**	B	C=O	O	1	C_6H_5	>300 [0.5]	>300 [0.5]		>30 [0.25]	>30 [0.25–4.0]	
(*R*)-**11**	C	C=O	NH	0	C_6H_5	>30, <100 [0.5]	>30, <100 [0.5]		ND[h]	ND[h]	
(*R*)-**12**	C	C=O	NH	2	C_6H_5	10 [0.25] (8.3–14)	50 [0.25] (42–80)	5.0	<30 [0.25–2.0]	>30 [0.24–4.0]	
(*R*)-**13**	C	C=O	NH	3	C_6H_5	16 [0.25] 13–17	43 [0.25] (38–47)	2.7	<30 [0.25–0.5]	>30 [0.25–4.0]	
(*R*)-**14**[i]	C	C=O	NH	4	C_6H_5	11 [0.25] (9.9–13)	38 [0.25] (34–42)	3.5	>30 [0.25–1.0]	>30 [0.25–4.0]	
(*R*)-**15**[j,k]	D	C=O	NH	1	C_6H_4(2′-OCF_3)	9.2 [0.25] (7.7–11)	51 [0.25] (38–65)	5.5	33 [0.5] (27–44)	>500	>15
(*R*)-**16**[l]	D	C=O	NH	1	C_6H_4(3′-OCF_3)	7.1 [0.25] (6.3–8.1)	30 [0.25] (27–32)	4.2	10 [1.0] (7.5–14)	43 [1.0] (35–57)	4.3
(*R*)-**17**[m,n,o]	D	C=O	NH	1	C_6H_4(4′-OCF_3)	16 [0.25] (14–20)	84 [0.25] (67–109)	5.3	18 [1.0] (12–28)	>500	>27
(*R*)-**18**	E	C=O	NH	1	C_6H_{11}	>30, <100 [0.5]	>100, <300 [0.5]		~15 [0.25–1.0]	>30 [0.25–4.0]	
(*R*)-**56**	C+D	C=O	NH	2	C_6H_4(2′-F)	27 [0.25] (22–32)	79 [0.25] (71–84)	2.9	20 [4.0] (11–31)	>500	>25
(*R*)-**57**	C+D	C=O	NH	2	C_6H_4(3′-F)	20 [0.25] (17–22)	67 [0.25] (61–75)	3.4	ND[h]	ND[h]	
(*R*)-**58**	C+D	C=O	NH	2	C_6H_4(4′-F)	>10, <30	>30, <100		ND[h]	ND[h]	
(*R*)-**59**[m]	D	C=O	NH	1	C_6H_4(4′-F)	32 [0.25] (28–39)	97 [0.25] (88–105)	3.0	21 [0.5] (13–31)	>500	>24
(*R*)-**26**[p]						4.5 [0.5] (3.7–5.5)	27 [0.25] (26–28)	6.0	3.9 [2.0] (2.9–6.2)	>500	>120



						Mice (ip)[a]			Rat (po)[b]		
Cmpd No	Site(s)	X	Y	n	R	MES,[c] ED_{50}	Tox,[d] TD_{50}	PI[e]	MES,[c] ED_{50}	Tox,[f] TD_{50}	PI[e]
phenytoin[q]						9.5 [2.0] (8.1–10)	27 [0.25] (26–28)	2.8	30 [4.0] (22–39)	>3000	>100
phenobarbital[q]						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[q]						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. 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] 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.

[c] MES = maximal electroshock seizure test.

[d] TD_{50} value determined from the rotorod test.

[e] PI = protective index (TD_{50}/ED_{50}).

[f] Tox = behavioral toxicity.

[g] Ref 6.

[h] ND = not determined.

[i] 6 Hz (32 mA) ED_{50} = 24 mg/kg [0.25 h] (17–29).

[j] 6 Hz (32 mA) ED_{50} = < 100 mg/kg;

[k] Formalin ED_{50} = 9.2 mg/kg [0.25 h].

[l] 6 Hz (32 mA) ED_{50} = 18 mg/kg [0.5 h] (14–24).

[m] Ref 8.

[n] 6 Hz (32 mA) ED_{50} = 28 mg/kg [0.5 h] (18–40).

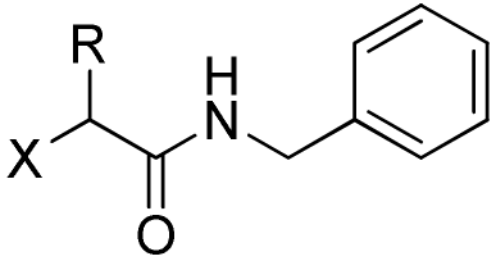
[o] Formalin ED_{50} = 16 mg/kg [0.25 h].

[p] Ref 11.

[q] Ref 7.

Table 2

Pharmacological activities of C(2)-substituted *N*-benzyl butanamides (Site F) in mice (mg/kg)[a] and rats (mg/kg)[b] and primary (PAAD), secondary (SAAD), and tertiary (TAAD) amino acid derivatives of (*R*)-*N*-benzyl 2-amino-3-methylbutanamide (Site F′) in mice (mg/kg)[a]



				Mice (ip)[a]			Rat (po)[b]		
Cmpd No.	**Site**	**R**	**X**	**MES,[c] ED_{50}**	**Tox,[d] TD_{50}**	**PI[e]**	**MES,[c] ED_{50}**	**Tox,[f] TD_{50}**	**PI[e]**
(*R*)-**19**[g]	F	CH_2CH_3	NH_2	18 [0.25] (10–25)	80 [0.25] (65–95)	4.4	11 [2.0] (7.8–16)	>500	>45
20	F	CH_2CH_3	H	>30, <100 [0.5]	>100, <300 [0.5]		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*,*S*)-**21**[h]	F	CH_2CH_3	CH_3	56 [0.25] (45–69)	165 [0.25] (148–180)	2.9	51 [0.5] (35–71)	>500	>9.8
(*S*)-**21**	F	CH_2CH_3	CH_3	>100, <300 [0.5]	~300 [0.5]		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**22**	F	CH_2CH_3	OH	>30, <100 [0.5]	>100, <300 [0.5]		>30 [0.25–4.0]	>30 [0.25–4.0]	
(*R*)-**3**[g]	F′	$CH(CH_3)_2$	NH_2	15 [0.25] (13–18)	70 [0.25] (63–80)	4.7	11 [0.25] (9.1–13)	>500	>45
(*R*)-**23**[i]	F′	$CH(CH_3)_2$	$N(H)CH_3$	25	ND[j]		ND[j]	ND[j]	
(*R*)-**24**	F′	$CH(CH_3)_2$	$N(CH_3)_2$	>30, <100 [0.5]	>100, <300 [0.5]		ND[j]	ND[j]	

[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. 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] 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.

[c] MES = maximal electroshock seizure test.

[d] TD_{50} value determined from the rotorod test.

[e] PI = protective index (TD_{50}/ED_{50}).

[f] Tox = behavioral toxicity.

[g] Ref 6.

[h] 6 Hz (32 mA) = 63 mg/kg.

[i] 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 and were determined 30 min after ip administration.

[j] ND = not determined.