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Design and biological evaluation of phenyl-substituted analogs of β-phenylethylidenehydrazine

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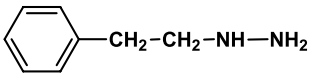
Abstract—β-Phenylethylidenehydrazine (PEH) has been demonstrated previously to be an inhibitor of γ-aminobutyric acid transaminase (GABA-T) and to cause a marked increase in rat brain levels of GABA, a major neurotransmitter. A group of PEH analogs, possessing a variety of substituents (Me, OMe, Cl, F, and CF_3) at the 2-, 3-, and 4-positions of the phenyl ring, were synthesized for evaluation as inhibitors of GABA-T. The details of the synthesis and chemical characterization of the analogs are described. Preliminary in vitro screening for GABA-T inhibition showed that all the analogs possessed activity against this enzyme, although substitution of CF_3 at the 2- and 4-positions caused reduced activity. One of the drugs, 4-fluoro-β-phenylethylidenehydrazine, was investigated further ex vivo, where it was shown to inhibit GABA-T, elevate brain levels of GABA, and decrease levels of glutamine, similar to the profile observed previously for PEH.

1. Introduction

Phenelzine (PLZ) (Fig. 1) is a monoamine oxidase (MAO)-inhibiting antidepressant, which is also effective for treating panic disorder and social anxiety disorder. PLZ is also an inhibitor of the γ-aminobutyric acid transaminase (GABA-T) and causes a marked elevation of brain levels of GABA in rats and gerbils.[1–3]

Because of its GABAergic properties, PLZ was tested in our laboratories to verify if it had neuroprotective properties similar to those of other GABAergic drugs.[4–8] PLZ was shown to reduce neuronal cell death if given prior to or within a few hours of induction of global ischemia in gerbils (an animal model of stroke).[3,9] These findings suggested that PLZ should be investigated further for its effects on stroke and other neurodegenerative disorders. However, because PLZ inhibits MAO irreversibly, there is the potential danger of adverse interactions of PLZ with foods containing sympathomimetic amines such as tyramine or with drugs such as cold preparations, which may contain sympathomimetic amines; the possibility of such interactions has severely restricted its use as a psychiatric drug. Studies by us and others[1,10] have provided indirect evidence for a metabolite of PLZ that might contribute to the GABA-elevating action of PLZ. We have synthesized one such putative metabolite, namely β-phenylethylidenehydrazine (PEH) (Fig. 1), and found that it retained the GABAergic actions of PLZ but had minimal effect on MAO,[11] suggesting that it could be an effective neuroprotective drug without the adverse effects of PLZ. To investigate the structure–activity relationships with PEH, we have prepared a number of phenyl ring-substituted analogs and conducted preliminary studies on their effects on



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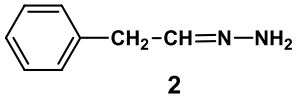

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Figure 1. Structures of phenelzine (**1**) and β-phenylethylidenehydrazine (**2**).

Keywords: β-Phenylethylidenehydrazine; γ-Aminobutyric acid; GABA transaminase; Glutamine.
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GABA metabolism. One of these drugs, 4-FPEH, was chosen for initial ex vivo studies on GABA-T activity and levels of GABA and related amino acids in rat brain since a substituent in the 4-position of the ring would be expected to reduce the likelihood of ring hydroxylation, perhaps increasing the availability of the drug in the brain. In addition, replacement of a hydrogen atom by a fluorine atom is known to enhance the pharmacological activity of many classes of drugs.

2. Results

A group of β-phenylethylidenehydrazines (**4a**–**p**), possessing a variety of substituents (Me, OMe, Cl, F, and CF_3) at the 2-, 3-, and 4-positions of the phenyl ring, were synthesized in 67–96% yield by condensation of the respective phenylacetaldehydes (**3a**–**p**) with hydrazine hydrate using a procedure similar to that reported for the preparation of the parent compound β-phenylethylidenehydrazine (**2**)[11,12] (Fig. 2).

Compounds **4a**–**p** were obtained as a mixture of the (*E*)-**4** and (*Z*)-**4** stereoisomers, in a ratio of approximately 3:1, which differ in stereochemistry about the C=N moiety. The ^{1}H NMR spectra for compounds **4a**–**p** exhibited dual resonances for the methylene (CH_2), methine (=C*H*), and amino (NH_2) groups. In this regard, the =C*H* and CH_2 resonances were deshielded, and the NH_2 resonance was shielded, in the (*E*)-isomer relative to the (*Z*)-isomer. These shielding effects are consistent with those reported for acetaldehyde hydrazones.[13]

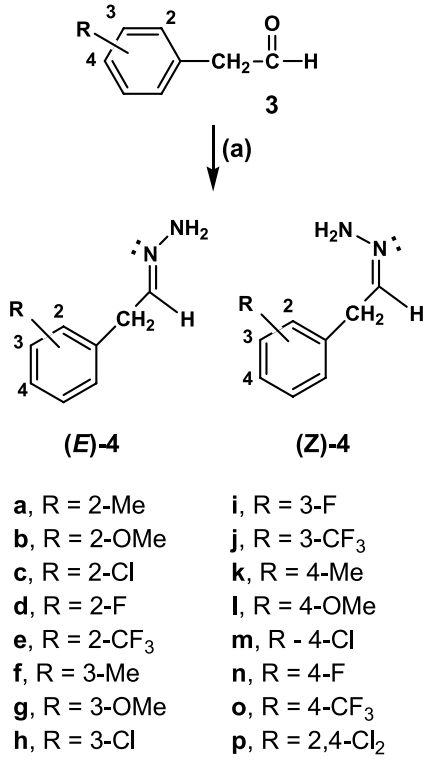

Figure 2. Reagents and conditions: (a) $H_2NNH_2{\cdot}H_2O$, 25 °C, 2 h (**4a**–**b**, **d**, and **f**–**o**), or 110 °C, 2 h (**c**, **e**, and **p**).

Table 1. Inhibition of GABA-T in vitro

Compound	R	% Inhibition of GABA-T[a]
PEH	H	75.4 ± 2.7
4a	2-Me	51.3 ± 0.6
4b	2-OMe	60.1 ± 0.4
4c	2-Cl	56.1 ± 2.8
4d	2-F	62.9 ± 0.2
4e	2-CF_3	46.3 ± 1.8
4f	3-Me	75.4 ± 0.2
4g	3-OMe	77.6 ± 0.7
4h	3-Cl	74.9 ± 0.1
4i	3-F	72.5 ± 0.3
4j	3-CF_3	76.0 ± 0.3
4k	4-Me	70.4 ± 0.4
4l	4-OMe	66.6 ± 0.9
4m	4-Cl	54.2 ± 0.2
4n	4-F	61.6 ± 0.7
4o	4-CF_3	33.2 ± 2.4
4p	2,4-Cl_2	59.0 ± 0.7

All drugs were present at a concentration of 10^{-4} M.
[a] Values are presented as means (% of control) ± standard error of the mean ($n = 4$).

The ratio of the (*E*)- and (*Z*)-stereoisomers **4a**–**p** was calculated from the integrals for the dual CH_2 resonances in the ^{1}H NMR spectrum.

The ability of this group of β-phenylethylidenehydrazines (**4a**–**p**) to inhibit GABA-T was determined using an in vitro assay, and the results are summarized in Table 1.

The in vitro screening data acquired indicate that all the analogs have the ability to inhibit GABA-T, but none was more effective than PEH at the concentration (10^{-4} M) tested. As illustrated in Figures 3 and 4, administration of 4-FPEH (**4n**) to rats was associated with time-dependent elevations in brain levels of GABA as well as inhibition of GABA-T.

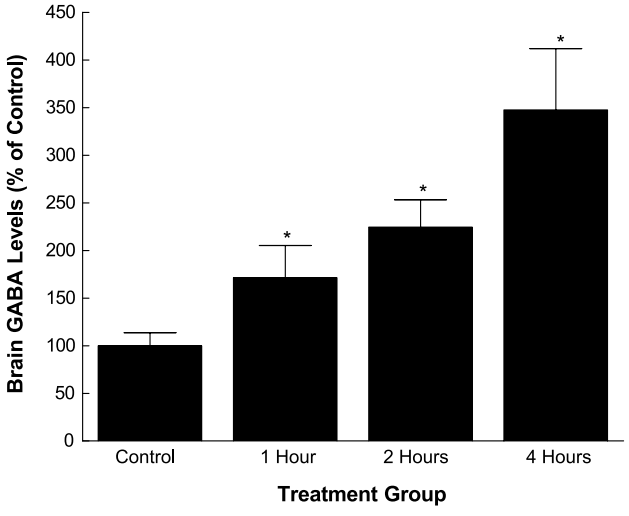

Figure 3. Brain GABA levels (mean ± SEM) in rats at varying times following a single injection of 4-FPEH (30 mg/kg, ip). Mean control (vehicle-treated) levels were 255 ± 50 μg/g ($n = 15$). *$p < 0.05$ versus control group. $n = 4$–5 animals per group.

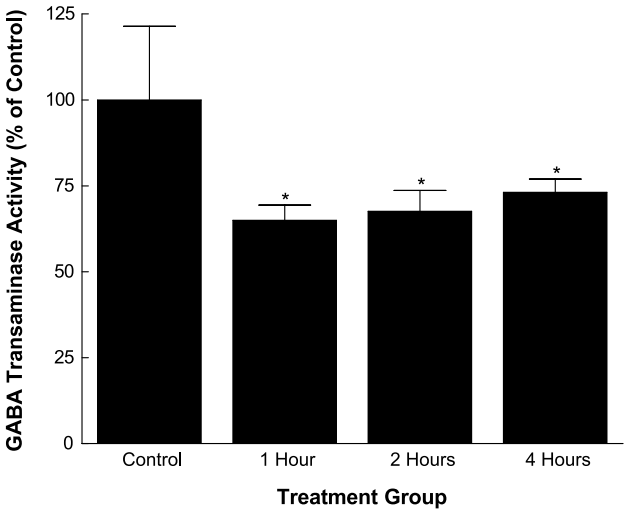

Figure 4. Inhibition of GABA-T (% of control, mean ± SEM) following a single injection of 4-FPEH (30 mg/kg, ip). *$p < 0.05$ versus control group. $n = 4$–8 animals per group.

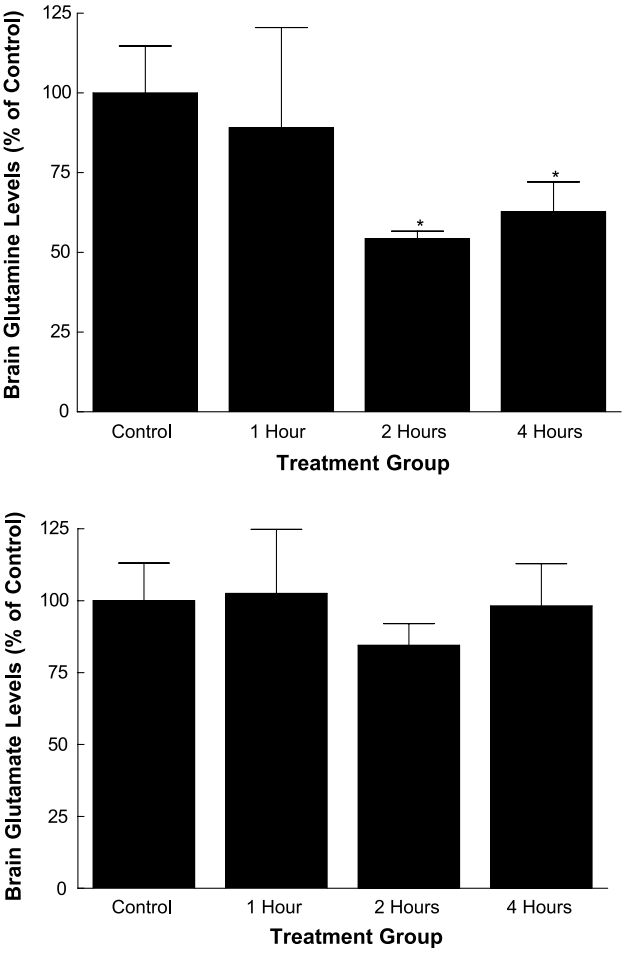

Figure 5. Brain glutamate and glutamine levels (mean ± SEM) in rats at varying times following a single injection of 4-FPEH (30 mg/kg, ip). Mean control (vehicle-treated) levels for glutamate and glutamine were 1924 ± 365 μg/g ($n = 14$) and 623 ± 148 μg/g ($n = 14$), respectively. *$p < 0.05$ versus control group. $n = 3$–5 animals per group.

Drug treatment was also associated with a significant reduction in brain glutamine content, but with no significant changes in brain glutamate levels (Fig. 5).

3. Discussion

While the in vitro screening data acquired indicate that all the analogs have the ability to inhibit GABA-T, compounds having a C-3 phenyl substituent (**4f–j**) were always more efficacious than the respective isomeric C-2 (**4a–e**) or C-4 (**4k–o**) compounds. The addition of a CF_3 group in the C-2 and C-4 positions reduced GABA-T-inhibiting activity rather markedly (Table 1).

The observed elevation in brain GABA levels following the administration of 4-FPEH (**4n**) (Fig. 3) is explained, at least in part, by inhibition of GABA-T (Fig. 4). The reason for the reduction in brain glutamine levels combined with maintenance of brain glutamate levels is not yet known but it is possible that these effects are a result of inhibition of glutamine synthetase concurrent with continued glutaminase activity. Such actions will now be studied.

The effects of 4-FPEH (**4n**) on GABA-T and levels of GABA, glutamate and glutamine are similar in direction and magnitude to those seen by Paslawski et al.[11] with the parent compound, PEH; similar results have now been obtained in gerbil brain (Sowa et al., unpublished). Based on the findings in vitro with all of the analogs synthesized and ex vivo with 4-FPEH, several of these analogs are now being investigated for their effects on brain levels of GABA, glutamate, and glutamine in rat and gerbil brain, and their actions in the global ischemia model of stroke.

4. Experimental

4.1. Materials

The following chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA): GABA, glutamate, glutamine, alanine, α-ketoglutaric acid, 2-aminoethylisothiouronium, and tri-*n*-octylamine. *o*-Phthalaldehyde reagent solution was obtained from Pierce Chemicals (Rockford, IL, USA).

4.2. Spectroscopy

Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (NH_2) was confirmed by the addition of D_2O. ^{13}C NMR were acquired using the *J* modulated spin-echo technique where methyl and methine carbon resonances appear as positive peaks, and methylene and quaternary carbon resonances appear as negative peaks. Silica gel column chromatography was performed using silica gel (70–230 mesh) purchased from Silicycle (Quebec, Canada). The phenylacetalde-

hydes **3a**–**p** were prepared by oxidation of the corresponding phenylethyl alcohols using dipyridine chromium (VI) oxide (Collin's reagent) in dry dichloromethane at 25 °C according to reported procedures.[14,15] 4-Trifluoromethyl- and 2,4-dichlorophenethyl alcohol, which were not commercially available from Aldrich, were prepared by the lithium aluminum hydride reduction of the respective 4-trifluoromethyl- and 2,4-dichlorophenylacetic acids using reported procedures.[16,17] All other reagents were purchased from Aldrich Chemicals (Milwaukee, WI).

4.3. General procedure for the syntheses of phenyl-substituted analogs of β-phenylethylidenehydrazine (4a–p)

The phenylacetaldehyde (**3a**–**p**, 1 mmol) was added dropwise to a solution of hydrazine monohydrate (10 mmol) with vigorous stirring at 25 °C, and the reaction was allowed to proceed with stirring for 2 h at 25 °C for the preparation of **4a**–**b**, **d**, and **f**–**o**, or heating at 110 °C for 2 h for the preparation of **4c**, **e**, and **p**. Chloroform (5 mL), and then water (3 mL), was added to the reaction mixture at 25 °C. This mixture was extracted with chloroform (3 × 5 mL), the combined chloroform extracts were washed with water (2 × 1 mL), and the chloroform fraction was dried (Na_2SO_4). Removal of the solvent in vacuo at 25 °C afforded the respective β-phenylethylidenehydrazine products **4a**–**p** as a pale yellow oil that consisted of a mixture of (*E*)-**4** and (*Z*)-**4** stereoisomers. Products **4a**–**p** were stored at −78 °C prior to their use in biological studies. The % chemical yield, (*E*):(*Z*) ratio at 25 °C as determined from the ^{1}H NMR integrals for the CH_2 resonances of the two isomers, and spectral data (IR, ^{1}H NMR, and ^{13}C NMR) for compounds **4a**–**p** are listed below.

4.3.1. [2-(2-Methylphenyl)ethylidene]hydrazine (4a). Yield, 96%; (*E*):(*Z*) = 72:28; IR (film): *v* 3382, 3214 (NH_2), 1649 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.16–7.21 (m, 4H, phenyl hydrogens), 7.13 (t, *J* = 5.7 Hz, 1H, C*H*=N), 5.15 (br s, 2H, NH_2), 3.52 (d, *J* = 5.7 Hz, 2H, CH_2CH), 2.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 144.06 (*C*H=N), 136.50, 135.55 (phenyl C-1, C-2), 130.21, 129.26 (phenyl C-3, C-6), 126.69, 126.00 (phenyl C-4, C-5), 36.51 (*C*H_2CH), 19.47 (*C*H_3); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.16–7.21 (m, 4H, phenyl hydrogens), 6.67 (t, *J* = 4.8 Hz, 1H, C*H*=N), 5.35 (br s, 2H, NH_2), 3.44 (d, *J* = 4.8 Hz, 2H, CH_2CH), 2.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 143.47 (*C*H=N), 136.50, 135.55 (phenyl C-1, C-2), 130.37, 128.97 (phenyl C-3, C-6), 126.92, 126.22 (phenyl C-4, C-5), 30.55 (*C*H_2CH), 19.47 (*C*H_3).

4.3.2. [2-(2-Methoxyphenyl)ethylidene]hydrazine (4b). Yield, 88%; (*E*):(*Z*) = 64:36; IR (film): *v* 3381, 3199 (NH_2), 1597 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.15–7.28 (m, 3H, phenyl H-4, H-6, C*H*=N), 6.88–6.95 (m, 2H, phenyl H-3, H-5), 5.13 (br s, 2H, NH_2), 3.84 (s, 3H, OCH_3), 3.52 (d, *J* = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): *δ* 157.19 (C-2), 144.93 (*C*H=N), 130.17 (C-6), 127.82 (C-4), 125.86 (C-1), 120.53 (C-5), 110.29 (C-3), 55.30 (O*C*H_3), 33.30 (*C*H_2CH); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.15–7.28 (m, 2H, phenyl H-4, H-6), 6.88–6.95 (m, 2H, phenyl H-3, H-5), 6.69 (t, *J* = 5.1 Hz, 1H, C*H*=N), 5.42 (br s, 2H, NH_2), 3.84 (s, 3H, OCH_3), 3.46 (d, *J* = 5.1 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): *δ* 157.19 (C-2), 144.20 (C*H*=N), 130.06 (C-6), 128.03 (C-4), 125.86 (C-1), 120.67 (C-5), 110.39 (C-3), 55.30 (O*C*H_3), 27.45 (*C*H_2CH).

4.3.3. [2-(2-Chlorophenyl)ethylidene]hydrazine (4c). Yield, 82%; (*E*):(*Z*) = 70:30; IR (film): *v* 3369, 3207 (NH_2), 1616 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.35–7.42 (m, 1H, H-3), 7.15–7.26 (m, 4H, H-4, H-5, H-6, C*H*=N), 5.20 (br s, 2H, NH_2), 3.64 (d, *J* = 5.4 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): *δ* 142.89 (*C*H=N), 139.30 (C-1), 135.36 (C-2), 130.79 (C-6) 129.45 (C-3), 128.03 (C-4), 126.92 (C-5), 36.49 (*C*H_2CH); (*Z*)-isomer, ^{1}H NMR $CDCl_3$): *δ* 7.35–7.42 (m, 1H, H-3), 7.15–7.26 (m, 3H, H-4, H-5, H-6), 6.67 (t, *J* = 4.5 Hz, 1H, C*H*=N), 5.40 (br s, 2H, NH_2), 3.57 (d, *J* = 4.5 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): *δ* 142.20 (*C*H=N), 139.30 (C-1), 135.36 (C-2), 130.38 (C-6), 129.66 (C-3), 128.29 (C-4), 127.09 (C-5), 30.55 (*C*H_2CH).

4.3.4. [2-(2-Fluorophenyl)ethylidene]hydrazine (4d). Yield, 71%; (*E*):(*Z*) = 73:27; IR (film): *v* 3368, 3214 (NH_2), 1622 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.01–7.27 (m, 5H, phenyl hydrogens, C*H*=N), 5.20 (br s, 2H, NH_2), 3.54 (d, *J* = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): *δ* 160.84 (d, $^1J_{C,F}$ = 246.1 Hz, C-2), 143.07 (*C*H=N), 130.85 (d, $^3J_{C,F}$ = 4.39 Hz, C-6), 128.32 (d, $^3J_{C,F}$ = 8.79 Hz, C-4), 124.34 (d, $^2J_{C,F}$ = 15.38 Hz, C-1), 124.10 (d, $^4J_{C,F}$ = 3.3 Hz, C-5), 115.27 (d, $^2J_{C,F}$ = 21.97 Hz, C-3), 32.07 (*C*H_2CH); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.01–7.27 (m, 4H, phenyl hydrogens), 6.70 (t, *J* = 4.5 Hz, 1H, C*H*=N), 5.40 (br s, 2H, NH_2), 3.49 (d, *J* = 4.5 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): *δ* 160.84 (d, $^1J_{C,F}$ = 246.1 Hz, C-2), 142.32 (*C*H=N), 130.52 (d, $^4J_{C,F}$ = 3.29 Hz, C-6), 128.56 (d, $^3J_{C,F}$ = 8.78 Hz, C-4), 124.34 (d, $^2J_{C,F}$ = 15.38 Hz, C-1), 124.32 (d, $^4J_{C,F}$ = 3.3 Hz, C-5), 115.48 (d, $^2J_{C,F}$ = 20.88 Hz, C-3), 25.79 (*C*H_2CH).

4.3.5. [2-(2-Trifluoromethylphenyl)ethylidene]hydrazine (4e). Yield, 91%; (*E*):(*Z*) = 73:27; IR (film): *v* 3369, 3201 (NH_2), 1609 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.30–7.65 (m, 4H, phenyl hydrogens), 7.14 (t, *J* = 5.4 Hz, 1H, C*H*=N), 5.20 (br s, 2H, NH_2), 3.69 (d, *J* = 5.4 Hz, 2H, CH_2CH); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.30–7.65 (m, 4H, phenyl hydrogens), 6.64 (t, *J* = 4.5 Hz, 1H, C*H*=N), 5.35 (br s, 2H, NH_2), 3.63 (d, *J* = 4.5 Hz, 2H, CH_2CH).

4.3.6. [2-(3-Methylphenyl)ethylidene]hydrazine (4f). Yield, 67%; (*E*):(*Z*) = 72:28; IR (film): *v* 3373, 3212 (NH_2), 1607 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.12–7.29 (m, 2H, H-5, C*H*=N), 7.07–7.09 (m, 3H, H-2, H-4, H-6), 5.20 (br s, 2H, NH_2), 3.48 (d, *J* = 5.7 Hz, 2H, CH_2CH), 2.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 144.81 (*C*H=N), 138.16, 137.18 (C-1, C-3), 129.51 (C-2), 128.42, 127.23 (C-4, C-5), 125.75 (C-6), 38.73 (*C*H_2CH), 21.32 (*C*H_3); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): *δ* 7.12–7.29 (m, 1H, H-5), 6.98–7.09 (m, 3H, H-2, H-4, H-6), 6.77 (t, *J* = 4.8 Hz, 1H, C*H*=N), 5.31 (br s, 2H, NH_2), 3.43 (d, *J* = 4.8 Hz, 2H, CH_2CH), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 144.06 (*C*H=N), 138.42,

136.44 (C-1, C-3), 129.29 (C-2), 128.64, 127.37 (C-4, C-5), 125.52 (C-6), 32.34 (CH_2CH), 21.32 (CH_3).

4.3.7. [2-(3-Methoxyphenyl)ethylidene]hydrazine (4g). Yield, 74%; (*E*):(*Z*) = 73:27; IR (film): *v* 3385, 3205 (NH_2), 1600 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.21–7.27 (m, 1H, H-5), 7.16 (t, J = 5.7 Hz, 1H, C*H*=N), 6.77–6.82 (m, 3H, H-2, H-4, H-6), 5.16 (br s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 3.49 (d, J = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 159.71 (C-3), 144.42 (*C*H=N), 138.87 (C-1), 129.50 (C-5), 121.08 (C-6), 114.38 (C-2), 111.95 (C-4), 55.13 (O*C*H$_3$), 38.84 (CH_2CH); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.21–7.27 (m, 1H, H-5), 6.77–6.82 (m, 4H, C*H*=N, H-2, H-6, H-6), 5.28 (br s, 2H, NH_2), 3.81 (s, 3H, OCH_3), 3.45 (d, J = 4.8 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 159.71 (C-3), 143.68 (*C*H=N), 138.87 (C-1), 129.74 (C-5), 120.83 (C-6), 114.25 (C-2), 112.04 (C-4), 55.13 (O*C*H$_3$), 32.46 (CH_2CH).

4.3.8. [2-(3-Chlorophenyl)ethylidene]hydrazine (4h). Yield, 95%; (*E*):(*Z*) = 73:27; IR (film): *v* 3361, 3203 (NH_2), 1602 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.08–7.25 (m, 5H, phenyl hydrogens, C*H*=N), 5.20 (br s, 2H, NH_2), 3.49 (d, J = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 143.43 (*C*H=N), 139.37 (C-1), 134.32 (C-3), 129.76 (C-5), 128.89 (C-2), 126.96, 126.73 (C-4, C-6), 38.41 (CH_2CH); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.08–7.25 (m, 4H, phenyl hydrogens), 6.72 (t, J = 4.5 Hz, 1H, C*H*=N), 5.30 (br s, 2H, NH_2), 3.45 (d, J = 4.5 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 142.60 (*C*H=N), 139.37 (C-1), 134.32 (C-3), 130.02 (C-5), 128.71 (C-2), 126.96, 126.73 (C-4, C-6), 32.02 (CH_2CH).

4.3.9. [2-(3-Fluorophenyl)ethylidene]hydrazine (4i). Yield, 85%; (*E*):(*Z*) = 73:27; IR (film): *v* 3375, 3207 (NH_2), 1622 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.23–7.31 (m, 1H, H-5), 7.15 (t, J = 5.4 Hz, 1H, C*H*=N), 6.90–7.02 (m, 3H, H-2, H-4, H-6), 5.21 (br s, 2H, NH_2), 3.51 (d, J = 5.4 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 162.87 (d, $^1J_{C,F}$ = 246.1 Hz, C-3), 143.56 (*C*H=N), 139.87 (d, $^3J_{C,F}$ = 7.69 Hz, C-1), 129.96 (d, $^3J_{C,F}$ = 7.69 Hz, C-5), 124.40 (d, $^4J_{C,F}$ = 2.2 Hz, C-6), 115.67 (d, $^2J_{C,F}$ = 20.88 Hz, C-2), 113.43 (d, $^2J_{C,F}$ = 20.88 Hz, C-4), 38.47 (CH_2CH); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.23–7.31 (m, 1H, H-5), 6.90–7.02 (m, 3H, H-2, H-4, H-6), 6.73 (t, J = 5.1 Hz, 1H, C*H*=N), 5.34 (br s, 2H, NH_2), 3.47 (d, J = 5.1 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 162.87 (d, $^1J_{C,F}$ = 246.1 Hz, C-3), 142.70 (*C*H=N), 139.87 (d, $^3J_{C,F}$ = 7.69 Hz, C-1), 130.26 (d, $^3J_{C,F}$ = 7.69 Hz, C-5), 124.19 (d, $^4J_{C,F}$ = 3.3 Hz, C_6 aryl), 115.53 (d, $^2J_{C,F}$ = 20.87 Hz, C-2), 113.74 (d, $^2J_{C,F}$ = 20.88 Hz, C-4), 32.11 (CH_2CH).

4.3.10. [2-(3-Trifluoromethylphenyl)ethylidene]hydrazine (4j). Yield, 89%; (*E*):(*Z*) = 75:15; IR (film): *v* 3369, 3214 (NH_2), 1622 (CH=N)cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.39–7.55 (m, 4H, phenyl hydrogens), 7.17 (t, J = 5.7 Hz, 1H, C*H*=N), 5.24 (br s, 2H, NH_2), 3.58 (d, J = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 143.17 (C*H*=N), 138.33 (C-1), 133.36 (q, $^2J_{C,F}$ = 29.66 Hz, C-3), 132.22 (C-6), 128.96 (C-5), 125.48 (q, $^3J_{C,F}$ = 3.3, C-2), 124.02 (q, $^1J_{C,F}$ = 272.46 Hz, *C*F$_3$), 123.42 (q, $^3J_{C,F}$ = 3.3 Hz, C-4), 38.54 (CH_2CH); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.39–7.55 (m, 4H, phenyl hydrogens), 6.73 (t, J = 5.1 Hz, 1H, C*H*=N), 5.32 (br s, 2H, NH_2), 3.54 (d, J = 5.1 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 142.30 (*C*H=N), 138.33 (C-1), 133.36 (q, $^2J_{C,F}$ = 29.66 Hz, C-3), 131.93 (C-6), 129.26 (C-5), 125.32 (q, $^3J_{C,F}$ = 3.3 Hz, C-2), 124.02 (q, $^1J_{C,F}$ = 272.46 Hz, *C*F$_3$) 123.64 (q, $^3J_{C,F}$ = 3.3 Hz, C-4), 32.14 (CH_2CH).

4.3.11. [2-(4-Methylphenyl)ethylidene]hydrazine (4k). Yield, 85%; (*E*):(*Z*) = 73:27; IR (film): *v* 3373, 3207 (NH_2), 1614 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.12–7.16 (m, 5H, C*H*=N, phenyl hydrogens), 5.15 (br s, 2H, NH_2), 3.48 (d, J = 5.7 Hz, 2H, CH_2CH), 2.33 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 144.96 (*C*H=N), 134.17 (C-1, C-4), 129.22 (C-2, C-6), 128.65 (C-3, C-5), 38.38 (CH_2CH), 21.00 (CH_3); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.12–7.14 (m, 4H, phenyl hydrogens), 6.77 (t, J = 4.8 Hz, 1H, C*H*=N), 5.35 (br s, 2H, NH_2), 3.44 (d, J = 5.7 Hz, 2H, CH_2CH), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 144.32 (*C*H=N), 134.17 (C-1, C-4), 129.44 (C-2, C-6), 128.40 (C-3, C-5), 32.01 (CH_2CH), 21.00 (CH_3).

4.3.12. [2-(4-Methoxyphenyl)ethylidene]hydrazine (4l). Yield, 87%; (*E*):(*Z*) = 73:27; IR (film): *v* 3388, 3220 (NH_2), 1609 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.11–7.15 (m, 3H, H-2, H-6, C*H*=N), 6.84–6.89 (m, 2H, H-3, H-5), 5.15 (br s, 2H, NH_2), 3.78 (s, 3H, OCH_3), 3.45 (d, J = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 158.40 (C-4), 144.96 (*C*H=N), 129.69 (C-2, C-6), 129.23 (C-1), 113.93 (C-3, C-5), 55.19 (O*C*H$_3$), 37.87 (CH_2CH); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.11–7.15 (m, 2H, H-2 and H-6 aryl), 6.84–6.89 (m, 2H, H-3 and H-5), 6.74 (t, J = 4.8 Hz, 1H, C*H*=N), 5.33 (br s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 3.41 (d, J = 4.8 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 158.40 (C-4), 144.32 (*C*H=N), 129.44 (C-2, C-6), 129.23 (C-1), 114.15 (C-3, C-5), 55.19 (O*C*H$_3$), 31.48 (CH_2CH).

4.3.13. [2-(4-Chlorophenyl)ethylidene]hydrazine (4m). Yield, 83%; (*E*):(*Z*) = 75:25; IR (film): *v* 3365, 3182 (NH_2), 1601 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.22–7.32 (m, 3H, H-3, H-5, C*H*=N), 7.08–7.16 (m, 2H, H-2, H-6), 5.20 (br s, 2H, NH_2), 3.47 (d, J = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 143.74 (*C*H=N), 141.10 (C-1), 136.70 (C-4), 130.12 (C-2, C-6), 128.65 (C-3, C-5), 38.12 (CH_2CH); (*Z*)-isomer, ^1H NMR ($CDCl_3$): δ 7.22–7.32 (m, 2H, H-3, H-5), 7.08–7.16 (m, 2H, H-2, H-6), 6.70 (t, J = 4.8 Hz, 1H, C*H*=N), 5.32 (br s, 2H, NH_2), 3.43 (d, J = 4.8 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 142.70 (*C*H=N), 141.10 (C-1), 136.70 (C-4), 129.88 (C-2, C-6), 128.93 (C-3, C-5), 31.76 (CH_2CH).

4.3.14. [2-(4-Fluorophenyl)ethylidene]hydrazine (4n). Yield, 70%; (*E*):(*Z*) = 75:25; IR (film): *v* 3368, 3189 (NH_2), 1602 (CH=N) cm^{-1}; (*E*)-isomer, ^1H NMR ($CDCl_3$): δ 7.14–7.24 (m, 3H, H-2, H-6, C*H*=N), 6.97–7.06 (m, 2H, H-3, H-5), 5.18 (br s, 2H, NH_2), 3.48 (d, J = 5.7 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 161.64 (d, $^1J_{C,F}$ = 243.89 Hz, C-4), 144.23 (*C*H=N), 132.98

(d, $^4J_{C,F}$ = 2.19 Hz, C-1), 130.22 (d, $^3J_{C,F}$ = 7.69 Hz, C-2, C-6), 115.35 (d, $^2J_{C,F}$ = 20.88 Hz, C-3, C-5), 37.99 (CH_2CH); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): δ 7.14–7.24 (m, 2H, H-2, H-6), 6.97–7.06 (m, 2H, H-3, H-5), 6.73 (t, *J* = 4.8 Hz, 1H, C*H*=N), 5.28 (br s, 2H, NH_2), 3.44 (d, *J* = 4.8 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 161.64 (d, $^1J_{C,F}$ = 243.89 Hz, C-4), 143.55 (*C*H=N), 132.98 (d, $^4J_{C,F}$ = 2.19 Hz, C-1), 130.21 (d, $^3J_{C,F}$ = 7.69 Hz, C-2, C-6), 115.56 (d, $^2J_{C,F}$ = 20.88 Hz, C-3, C-5), 31.60 (CH_2CH).

4.3.15. [2-(4-Trifluoromethylphenyl)ethylidene]hydrazine (4o). Yield, 90%; (*E*):(*Z*) = 73:27; IR (film): *v* 3363, 3198 (NH_2), 1623 (C=N)cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): δ 7.55–7.61 (m, 2H, H-3 and H-5), 7.31–7.36 (m, 2H, H-2 and H-6), 7.16 (t, *J* = 5.4 Hz, 1H, C*H*=N), 5.29 (br s, 2H, NH_2), 3.57 (d, *J* = 5.4 Hz, 2H, CH_2CH); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): δ 7.55–7.61 (m, 2H, H-3 and H-5), 7.31–7.36 (m, 2H, H-2 and H-6), 6.72 (t, *J* = 4.8 Hz, 1H, C*H*=N), 5.39 (br s, 2H, NH_2), 3.53 (d, *J* = 4.8 Hz, 2H, CH_2CH).

4.3.16. [2-(2,4-Dichlorophenyl)ethylidene]hydrazine (4p). Yield, 71%; (*E*):(*Z*) = 72:28; IR (film): *v* 3369, 3201 (NH_2), 1589 (CH=N) cm^{-1}; (*E*)-isomer, ^{1}H NMR ($CDCl_3$): δ 7.16–7.42 (m, 4H, H-3, H-5, H-6, C*H*=N), 5.22 (br s, 2H, NH_2), 3.59 (d, *J* = 5.1 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 142.02 (*C*H=N), 134.58, 133.97, 133.03 (C-1, C-2, C-4), 131.52 (C-6), 129.21 (C-3), 127.15 (C-5), 35.87 (CH_2CH); (*Z*)-isomer, ^{1}H NMR ($CDCl_3$): δ 7.16–7.42 (m, 3H, H-3, H-5, H-6), 6.61 (t, *J* = 4.8 Hz, 1H, C*H*=N), 5.35 (br s, 2H, NH_2), 3.52 (d, *J* = 4.8 Hz, 2H, CH_2CH); ^{13}C NMR ($CDCl_3$): δ 141.30 (*C*H=N), 134.58, 133.97, 133.03 (C-1, C-2, C-4), 131.08 (C-6), 129.44 (C-3), 127.36 (C-5), 29.97 (CH_2CH).

4.4. Injection of animals

Male Sprague–Dawley rats (200–250 g) were injected intraperitoneally with 4-FPEH or vehicle (corn oil) and killed at various time intervals after injection by decapitation. Brains were immediately frozen in isopentane on solid carbon dioxide, removed to another set of containers, and frozen at −80 °C until the time of analysis. All procedures were approved by the Health Sciences Animal Welfare Committee, Faculty of Medicine and Dentistry, University of Alberta.

4.5. GABA transaminase assay

In the initial in vitro screen for GABA-T activity, a modification of the colorimetric method of Sethi (1993) was used. In the ex vivo study of homogenates from brains of rats treated with **4n** ([2-(4-fluorophenyl)ethylidine]-hydrazine; 4-FPEH), GABA-T activity was measured using the radiochemical procedure of Sterri and Fonnum,[18] as modified by McManus et al.[19] Briefly, homogenates were incubated with radiolabelled GABA in the presence of pyridoxal phosphate and the resultant products were isolated using a liquid anion exchanger (tri-*n*-octylamine). The radioactivity was then counted using a liquid scintillation counter (Beckman LS 6000SC).

4.6. HPLC assay for amino acids

Rat brain tissue was homogenized in 5 volumes of double-distilled water. The homogenates were then diluted in 5 volumes of 100% methanol, centrifuged, and the supernatants were further diluted in 10 volumes of double-distilled water. Once loaded into the HPLC, immediately prior to injection amino acids were derivatized to fluorescent thioalkyl-substituted isoindoles by reaction with *o*-phthaldialdehyde in alkaline medium, with 2-mercaptoethanol serving as a reducing agent.[20] Fluorescence emitted by the thioalkyl derivatives was detected using a Shimadzu RF10A fluorescence detector (excitation wavelength 260 nm and emission wavelength 455 nm) following elution of the derivatized amino acids from the column.

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