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N-Propynyl analogs of β-phenylethylidenehydrazines: Synthesis and evaluation of effects on glycine, GABA, and monoamine oxidase ☆

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ABSTRACT

A group of β-phenylethylidenehydrazines possessing a variety of substituents (Me, OMe, Cl, F, and CF_3) at the *ortho*-, *meta*-, or *para*-positions of the phenyl ring, in conjunction with either a *N*-bis-(2-propynyl) or a *N*-mono-(2-propynyl) moiety, were synthesized and compared to the novel neuroprotective drug β-phenylethylidenehydrazine (PEH) with regard to their ability to inhibit the enzymes GABA-transaminase (GABA-T) and monoamine oxidase (MAO)-A and -B in vitro in brain tissue. Two of the analogs synthesized (mono- and bis-*N*-propynylPEH) were also studied *ex vivo* in rats to compare their effects to those of PEH with regard to ability to inhibit GABA-T and MAO and to change brain levels of several important amino acids. Unlike PEH, none of the new drugs inhibited GABA-T in vitro at 10 or 100 μM, and all of the drugs (including PEH) were poor inhibitors (at 10 μM) of MAO-A and -B *in vitro*. The two analogs studied *ex vivo* inhibited GABA-T to a lesser extent than PEH, unlike PEH that did not elevate brain levels of GABA, and inhibited MAO-A and -B more potently than PEH. Interestingly, unlike PEH, the two analogs caused marked increases in brain levels of glycine; because of the current interest in drugs that increase glycine availability in the brain as potential antipsychotic drugs, these two analogs now warrant further investigation.

1. Introduction

β-Phenylethylidenehydrazine [PEH; Fig. 1(1)] is a putative metabolite of the antidepressant/antipanic/neuroprotective drug phenelzine [PLZ; Fig. 1(2)] and is thought to be formed by the action of monoamine oxidase (MAO) on PLZ.[1–4] PEH shares a number of neurochemical properties with PLZ, including (a) the inhibition of GABA-transaminase (GABA-T) activity;[5] (b) the ability to increase brain levels of GABA,[5,6] alanine and ornithine;[7] and (c) the ability to transiently decrease brain levels of glutamine.[5] Interestingly, inhibition of MAO prior to PLZ administration, presumably inhibiting the formation of PEH, prevents some of these effects, such as inhibition of GABA-T, and elevation of GABA and ornithine.[8–10] PEH formation may also contribute to some of the therapeutic effects of PLZ. For example, the anxiolytic properties of PLZ have been shown to be related to the drug's facilitatory effect on GABAergic transmission,[11] and given that the increase in GABA appears to be dependent upon PEH formation, it is reasonable to suggest that PEH may mediate the anxiolytic effects of PLZ. Furthermore, PLZ and PEH have been shown to be neuroprotective in animal models of global ischemia,[12,13] and while the mechanisms for this have not been elucidated, the ability of PLZ to reduce glutamatergic transmission,[14,15] the ability of PLZ (and most likely PEH) to sequester reactive aldehydes,[12] and the ability of both drugs to increase brain GABA[8,16–21] probably contribute to neuroprotection. Importantly, PEH differs from PLZ in that it does not appreciably inhibit MAO activity.[5] Given the strict dietary restrictions that are necessary for individuals taking PLZ due to potentially dangerous interactions between the drug and tyramine-rich

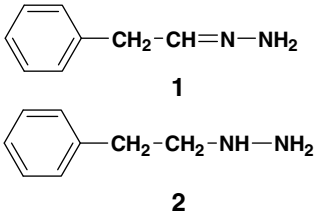

Figure 1. Structures of β-phenylethylidenehydrazine (**1**) and phenelzine (**2**).

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foods, PEH may be a useful alternative for conditions thought to involve GABAergic dysfunction and in which PLZ is effective (e.g., depression, social anxiety disorder, and panic disorder) but is not used as a first-line drug because of this adverse effect. Certainly, PEH should be further investigated as a therapeutic drug in and of itself.

In the present report, a group of β-phenylethylidenehydrazines possessing a variety of substituents (Me, OMe, Cl, F, and CF_3) at the *ortho*-, *meta*-, or *para*- positions of the phenyl ring, in conjunction with either a *N*-bis-(2-propynyl) (**7p**) or a *N*-mono-(2-propynyl) (**8p**) moiety, have been synthesized (Fig. 2) and compared to PEH with regard to their ability to inhibit GABA-T and MAO-A and -B in vitro. The choice of aryl substituents selected (Me, OMe, Cl, F, and CF_3) was based on the knowledge that these substituents are frequently present in drugs used to treat psychiatric and neurologic disorders. This group of substituents was selected to determine positional, steric, electronic, and lipophilic aryl substituent effects on neurochemical action(s) of the drugs of interest. In this regard, the presence of substituents in the phenyl ring may enhance pharmacological activity and/or reduce metabolism of the drugs. Removal of *N*-propynyl (*N*-propargyl) groups metabolically has been shown to occur readily in a number of arylalkylamine drug molecules.[22–34] Moreover, an effective prodrug has been produced by the addition of one or two propynyl groups to the biogenic amine β-phenylethylamine (PEA), increasing brain, liver and plasma levels of PEA when compared to administration of the non-propargylated form of the amine and prolonging its clearance from the body.[35,36] Since PEH appears to possess a number of potentially beneficial neurochemical properties, it would be of interest to see if the drugs synthesized here are effective prodrugs. Two of the analogs, **7p** and **8p** were compared to PEH with regard to their *ex vivo* effects on GABA-T, MAO, and levels of amino acids to determine their possible value as PEH prodrugs.

2. Results

2.1. Chemistry

A group of β-phenylethylidenehydrazines possessing a variety of substituents (Me, OMe, Cl, F, and CF_3) at the *ortho*-, *meta*-, or *para*-positions of the phenyl ring, in conjunction with either a *N*-bis-(2-propynyl) (**7**) or a *N*-mono-(2-propynyl) (**8**), moiety were synthesized by condensation of the respective phenylacetaldehydes (**6a–p**) with a mixture of 1,1-bis-(2-propynyl)hydrazine (**4**), and 1-(2-propynyl)hydrazine (**5**) in methanol at 25 °C, as illustrated in Figure 2. The *N*-bis-(2-propynyl) compounds **7** were obtained as the (*E*)-stereoisomer in 12–18% yield. In contrast, the *N*-mono-(2-propynyl) compounds **8** were isolated in 18–34% yield as a mixture of the **(*E*)-8** and **(*Z*)-8** stereoisomers, which differ in stereochemistry about the C=N moiety; the ratio of (*E*):(*Z*) isomers was in a range between 1.6:1 to 6:1. The **(*E*)-7**, and an inseparable mixture of the **(*E*)-8** and **(*Z*)-8**, compounds were separated by silica gel flash chromatography. The homogeneity of the *N*-bis-(2-propynyl) compounds **7** is supported by the fact that a single spot was observed in each case upon thin layer chromatography (TLC) using a Macherey–Nagel silica gel Polygram SIL G/UV_{254} plate (0.20 mm thickness) with a development solvent that provided reproducible, similar R_f values for the compounds of interest. The mixture of the **(*E*)-8** and **(*Z*)-8** isomers showed an inseparable spot under similar TLC conditions. The ^{1}H NMR spectra for the mixture of **(*E*)-8** and **(*Z*)-8** compounds exhibited dual resonances for the methylene (CH_2) and methine (=C*H*) groups. In this regard, the =C*H* and CH_2 resonances were deshielded in the (*E*)-isomer relative to the (*Z*)-isomer. These shielding effects are consistent with those reported for acetaldehyde hydrazones.[37] The single spot on TLC and the combination of both proton and carbon NMR spectra, accounting for all the hydrogen and carbon atoms in the molecule, provided reliable confirmation of homogeneity and structure. In

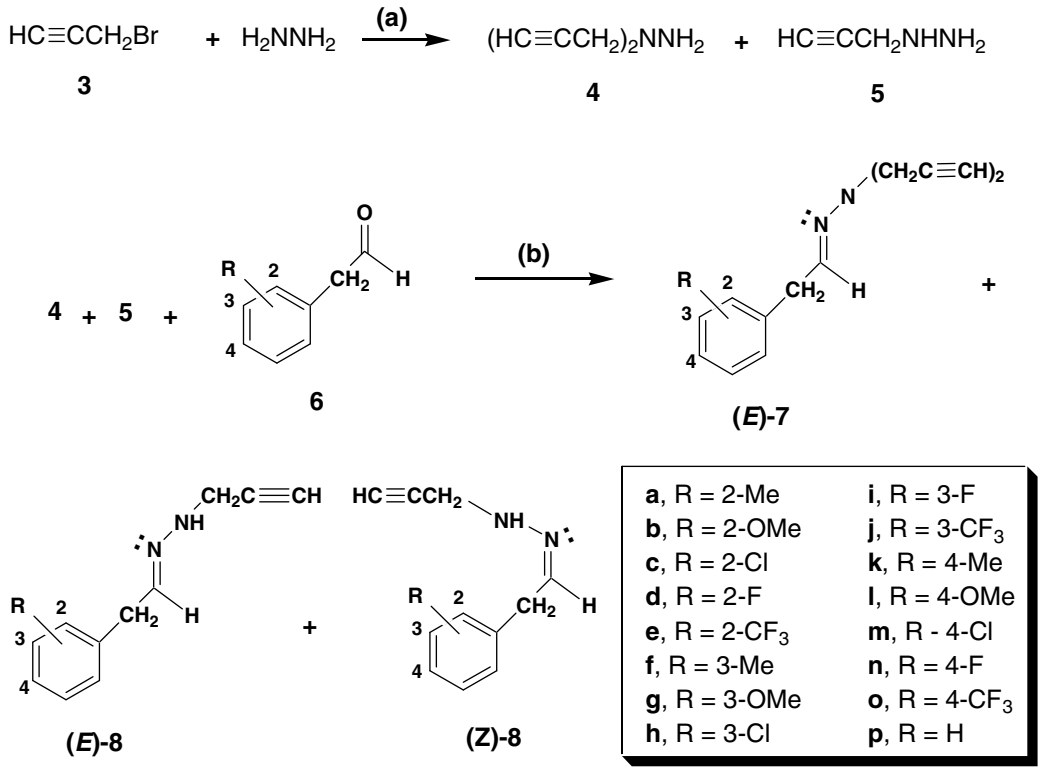

Figure 2. Reagents and conditions: (a) $H_2NNH_2 \cdot H_2O$, MeOH, reflux, 30 min; (b) MeOH, 25 °C, 2 h.

addition, **7p** and **8p**, which were studied ex vivo as well as in vitro for neurochemical/pharmacological activity, were investigated on HPLC with electrochemical detection. Studies with various mobile phases on an Atlantis dC18 column (3.0 × 100 mm) revealed a single peak in all cases.

2.2. GABA-T

In vitro, PEH inhibited GABA-T by approximately 50% at a concentration of 10 μM, while none of the analogs inhibited GABA-T at concentrations of either 10 μM or 100 μM (data not shown). Ex vivo, PEH significantly reduced GABA-T activity at all time points tested; activity was reduced to 66% of controls at 3 and 6 h post-injection, and activity remained inhibited 12 h after PEH injection (70% of control). The degree of GABA-T inhibition by PEH at this dose is consistent with previous data.[5] **7p** and **8p** also decreased GABA-T activity at 3 and 6 h following drug administration, although the inhibitory effect was significantly weaker than that observed following PEH administration (86% of control for **7p** at 3 and 6 h after injection, and 76% and 77% of control for **8p** at 3 and 6 h, respectively). GABA-T activity was not significantly different from control values 12 h after **7p** or **8p** injection. These results are shown in Figure 3.

2.3. MAO

In vitro, PLZ inhibited both MAO-A and -B by greater than 95%, while PEH had a much weaker effect on both MAO isoenzymes (26.7 ± 3.1% and 14.9 ± 6.6% inhibition (mean ± SEM, $n = 4$) for MAO-A and -B, respectively). Under the same conditions, none of the analogues tested inhibited MAO-A or MAO-B by more than 15%. PEH has been shown previously to have a weak, transient effect on MAO-A and -B activity ex vivo,[5] and PEH did not alter the activity of either MAO-A or -B in brain tissue taken from PEH-treated rats. At the same dose **7p** did not significantly alter MAO-A activity at any time point tested, and **8p** had a weak, transient effect on MAO-A, significantly reducing activity at 3 and 6 hours following drug administration to 78% and 70% of control values (i.e., 22% and 30% inhibition), respectively. In contrast, both **7p** and **8p** had relatively marked inhibitory effects on MAO-B activity, significantly reducing MAO-B activity at all time points tested. These effects were particularly pronounced at 6 and 12 h following drug injection, where MAO-B activity was reduced by **7p** to 58% and 25% of control at 6 and 12 h (respectively) and by **8p** to 24% and 29% of control at 6 and 12 h (respectively). These results are shown in Figure 4.

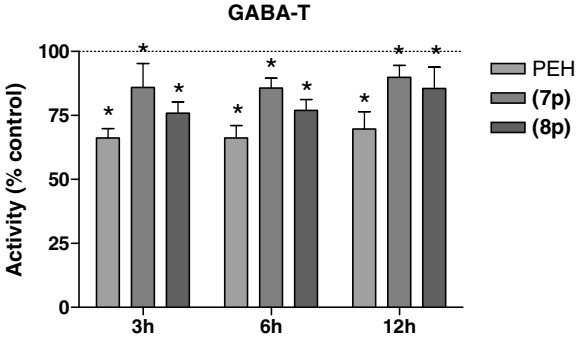

Figure 3. Rat whole brain activity of GABA-T (mean % control ± SEM) at 3, 6, and 12 h following i.p. injection of PEH (30 mg/kg), **7p** (47 mg/kg), or **8p** (38.5 mg/kg). $N = 4–5$ for each group. * indicates significantly different from vehicle controls ($P < 0.05$).

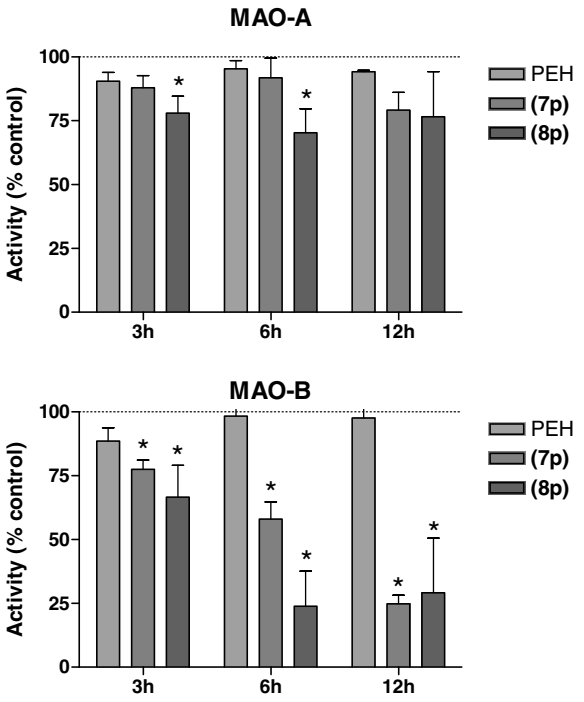

Figure 4. Rat whole brain activity of MAO-A and MAO-B (mean % control ± SEM) at 3, 6, and 12 h following i.p. injection of PEH (30 mg/kg), **7p** (47 mg/kg), or **8p** (38.5 mg/kg). $N = 3–6$ for each group. * indicates significantly different from vehicle controls ($P < 0.05$).

2.4. Amino acids

Consistent with our previous studies, the *ex vivo* studies demonstrated that PEH increased brain GABA and alanine levels, an effect which was significant at all three timepoints following drug administration. Elevations in brain GABA and alanine were greatest at 3 h following PEH injection, reaching 418% and 489% of control values, respectively. As expected, PEH did not significantly alter brain glutamate or glycine levels relative to controls. **8p** and **7p** administration did not significantly alter the levels of GABA, alanine or glutamate at any time point tested. However, interestingly **8p** and **7p** caused marked increases in brain glycine levels; **8p** significantly increased glycine at 3 and 6 h post-injection, peaking at 216% of control at 6 h, and **7p** increased glycine at 3, 6, and 12 h post-injection, peaking at 248% of control at 12 h. These results are shown in Figure 5.

3. Discussion

PEH is a putative metabolite of PLZ and is thought to contribute to some of PLZ's neurochemical and therapeutic properties. On its own, PEH has been shown to be a potent inhibitor of GABA-T, significantly elevating brain GABA levels, while its effects on MAO activity are weak and transient.[5] Its potential as a GABAergic agent warrants further investigation. In the present experiments, a number of PEH analogs substituted in the phenyl ring and by one or two propynyl groups at the terminal nitrogen were synthesized and tested for their effects on GABA-T and MAO in vitro. Two of the analogs (**7p** and **8p**) were also tested ex vivo for their effects on GABA-T, MAO-A and -B and amino acids.

The neurochemical properties of PEH and those of the analogs were quite dissimilar. It is well-established that PEH, like PLZ, is an inhibitor of GABA-T (the enzyme responsible for the breakdown of GABA) and causes marked, long-lasting increases in brain levels of GABA.[5] This elevation in GABA by PLZ has been found by us and

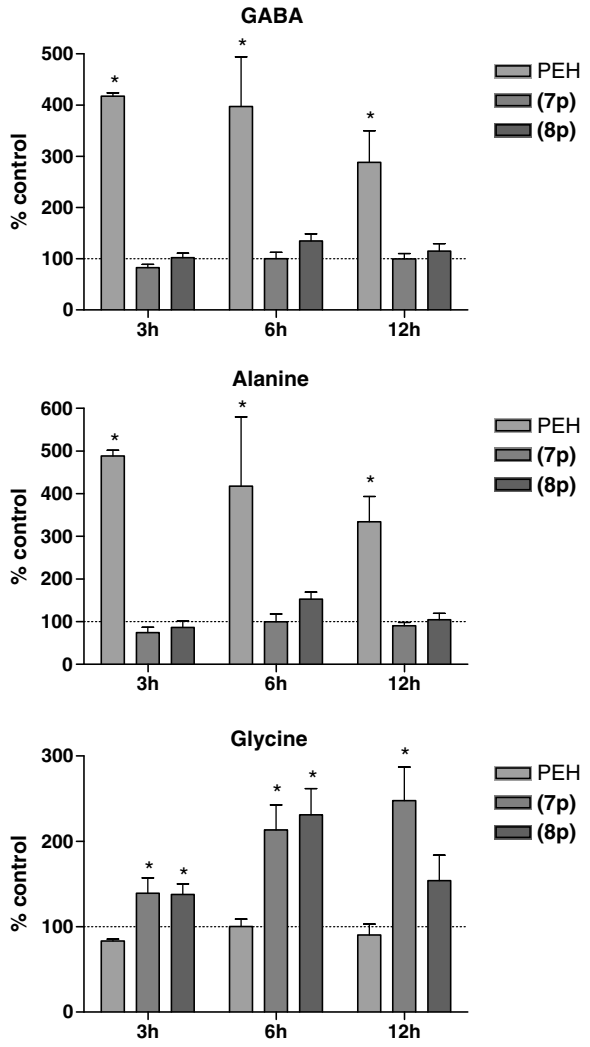

Figure 5. Rat whole brain GABA, alanine, and glycine levels (mean % control ± SEM) at 3, 6, and 12 h following i.p. injection of PEH (30 mg/kg), **7p** (47 mg/kg), or **8p** (38.5 mg/kg). $N = 3–5$ for each group. * indicates significantly different from vehicle controls ($P < 0.05$). Mean control values were 194.2 ± 11.5 μg/g tissue for GABA, 50.0 ± 3.4 μg/g tissue for alanine, and 56.4 ± 2.6 μg/g tissue for glycine.

others to occur at a relatively low inhibition of GABA-T.[8,10] While **7p** and **8p** also inhibited GABA-T, the inhibition at the equivalent dose tested was to a significantly lesser degree than the inhibition observed following administration of PEH itself, and was apparently insufficient to significantly elevate brain GABA levels. However, the fact that these two analogs caused some inhibition of GABA-T activity ex vivo while being inactive in vitro suggests that some metabolic conversion of them to PEH does occur ex vivo, and this possibility will now be investigated by measuring brain and liver levels of PEH after administration of the two analogs to rats intraperitoneally. PEH also elevated brain levels of alanine, while administration of **7p** and **8p** did not alter alanine levels. Given that the breakdown of alanine is achieved by the action of alanine transaminase (ALA-T), an enzyme structurally and functionally related to GABA-T, it has been suggested that PEH inhibits ALA-T in a similar manner to the inhibition of GABA-T, causing increased alanine levels. **7p** and **8p**, which are less potent at inhibiting GABA-T, would also be expected to be less potent than PEH in inhibiting ALA-T, resulting in the unaltered alanine levels observed here. None of the drugs had any effect on brain levels of glutamate. However, whereas PEH did not significantly alter glycine levels, both **7p** and **8p** caused marked increases in brain glycine levels. This finding is discussed further below.

Previous experiments in our laboratories have shown that PEH does not greatly affect the activity of MAO-A or -B. In the present experiments ex vivo, **7p** and **8p** had modest inhibitory effects on MAO-A at some (but not all) timepoints. However, unlike PEH, both **7p** and **8p** had relatively strong inhibitory effects on MAO-B, particularly 6 and 12 h after drug administration. This was unexpected, since neither **7p** nor **8p** caused any inhibition of MAO-A or -B in vitro at a concentration of 10 μM. Measurement of brain levels of these analogs in the future may shed some light on this matter. Dose–response and time–response studies are also now underway to determine if these inhibitory effects are reflected in changes in brain levels of the neurotransmitter amines dopamine, norepinephrine and/or 5-hydroxytryptamine (5-HT, serotonin), which are substrates for MAO. A greater degree of inhibition of MAO is required in order to produce marked increases in brain levels of these neurotransmitter amines[38,39] than is apparently the case with GABA-T and GABA.

Given that **7p** and **8p** exhibited such different neurochemical profiles from PEH itself, it is unlikely that the propargylated analogues act as efficient PEH prodrugs. This result was somewhat surprising, since a number of studies have demonstrated the extensive *N*-depropargylation of drugs such as (−)-deprenyl, rasagiline and pargyline,[22–34] and the utility of *N*-propynyl forms of β-phenylethylamine as prodrugs.[35,36] However, despite the fact that **7p** and **8p** seem unlikely to be suitable PEH prodrugs, these drugs have interesting neurochemical properties in their own right. They are inhibitors of MAO-B ex vivo, which is interesting given that other MAO-B inhibitors such as l-deprenyl and rasagiline have been reported to be useful in treatment of neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD). Inhibition of MAO-B could increase brain levels of dopamine in patients suffering from PD, and could counteract the increased MAO activity and the increased MAO mRNA expression observed in AD.[38] The neuroprotective abilities of the drugs mentioned above (l-deprenyl, rasagiline) are not completely understood, and are not thought to be solely related to their ability to inhibit MAO-B; they have in common with our analogs the presence of the *N*-propynyl moiety. Based on this evidence, **7p** and **8p** may also be interesting candidates for novel neuroprotective agents. We have previously prepared *N*-propynyl analogs of PLZ and found them to be weaker inhibitors of MAO than PLZ (but stronger than the analogs of PEH described in the present study) and to be reasonably potent at preventing norepinephrine depletion caused by the neurotoxin DSP-4.[39]

The most unexpected finding in the present experiments was the exciting observation that **7p** and **8p** markedly increased brain levels of glycine. Glycine acts as an inhibitory amino acid in the brain stem and spinal cord,[40] but is also a co-agonist at the glutamate NMDA receptor,[41] and thus exerts excitatory effects in other brain areas such as the cortex and hippocampus. Interestingly, NMDA receptor hypofunction is believed by many to contribute to the symptoms of schizophrenia.[42] Preclinical and clinical studies have consistently demonstrated that NMDA antagonists produce a range of symptoms characteristic of the illness.[42,43] While direct glycine agonists have been reported to be useful in treating negative and possibly cognitive symptoms of schizophrenia, they have been of limited clinical utility because of the high doses required and their relatively poor penetration of the blood–brain barrier.[43] However, indirect increases in brain glycine *via* inhibition of glycine transporters (proteins that remove glycine from the synapse) have shown substantial promise.[43] **7p** and **8p** could also be useful as therapeutic agents in schizophrenia, since they offer another indirect mechanism by which brain glycine is elevated (and therefore NMDA receptor activity possibly facilitated). The primary

route of glycine metabolism in animals involves the glycine cleavage system (GCS), a mitochondrial complex of four enzymes that is present in many vertebrate organs, including the brain. It is possible that **7p** and **8p** inhibit GCS activity, resulting in the dramatic increases in glycine observed here; we are currently investigating this possibility.

4. Conclusions

A number of *N*-mono-(2-propynyl) and *N*-bis-(2-propynyl) analogs of PEH with varying substituents in the phenyl ring were synthesized, characterized by TLC followed by proton and carbon NMR and tested for their ability to inhibit GABA-T, MAO-A, and MAO-B in vitro in rat brain tissue. Two of the compounds, **7p** and **8p** (*N*-bis-(2-propynyl)PEH and *N*-mono(2-propynyl)PEH) were studied further by investigating their effects on brain levels of amino acids and on activities of GABA-T, MAO-A, and MAO-B after intraperitoneal injection to evaluate their value as prodrugs of PEH, given that the *N*-propynyl group(s) in similar molecules has been shown to be removed metabolically *in vivo*. None of the analogs was particularly effective at inhibiting GABA-T, MAO-A, or MAO-B in vitro. In the ex vivo study in rats, neither **7p** or **8p** produced as strong effects on inhibition of GABA-T and on elevation of brain GABA as PEH, suggesting that they were not particularly effective prodrugs of PEH at the dose studied. Ex vivo, **7p** did not inhibit MAO-A while **8p** had a weak transient effect, but both drugs inhibited MAO-B at all time intervals studied, presumably because of the presence of the propynyl substituent. The most noteworthy effect of **7p** and **8p** was the considerable increase in brain levels of glycine (PEH produced no such increase). Glycine is a co-agonist at the excitatory NMDA glutamate receptor. The glutamatergic system has been proposed to be hypofunctional in schizophrenia, and administration of high doses of glycine to individuals with schizophrenia has been reported to improve the negative symptoms of this disorder.[43] **7p** and **8p** represent a means to elevate brain glycine without administering high doses of glycine, and this exciting effect is now being pursued further in our laboratories.

5. Experimental

5.1. Materials

The following chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA): GABA, glutamate, alanine, glycine, α-ketoglutaric acid, 2-aminoethylisothiouronium, tri-*n*-octylamine, *o*-phthalaldehyde, pyridoxal phosphate, and GABAase. *N*-isobutyryl-L-cysteine was purchased from Novabiochem (La Jolla, CA, USA), and ^{14}C-β-phenylethylamine, ^{14}C-5-hydroxytryptamine and ^{3}H-GABA were purchased from Perkin-Elmer (Waltham, MA, USA). All other reagents were purchased from Aldrich Chemicals (Milwaukee, WI, USA) or Fischer Scientific (Ottawa, ON, Canada).

5.2. 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 spectrophotometer. The assignment of exchangeable protons (N*H*) was confirmed by the addition of D_2O. ^{13}C NMR spectra 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). 1,1-bis-(2-Propynyl)hydrazine (**4**) and 1-(2-propynyl)hydrazine (**5**) were prepared by reaction of propargyl bromide (**3**) with hydrazine hydrate according to a patent procedure.[44] The phenylacetaldehydes **6a–n** 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.[45,46] 4-Trifluoromethylphenethyl alcohol, which was not commercially available from Aldrich, was prepared by the lithium aluminum hydride reduction of 4-trifluoromethylphenylacetic acid using reported procedures.[47,48] The ratio of the **(*E*)-8** and **(*Z*)-8** stereoisomers was calculated from the integrals for the dual C*H*$_2$ resonances in the ^{1}H NMR spectrum.[37]

5.3. General procedure for the syntheses of (*E*)-*N*-bis-(2-propynyl)-2-(2-, 3- and 4-substituted-phenyl)ethyidene]-hydrazines [(*E*)-7], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(2-, 3- and 4-substituted-phenyl)ethylidene]hydrazines [(*E*)-8 and (*Z*)-8] (Fig. 2)

The phenylacetaldehyde (**6a–p**, 1.9 mmol), and a mixture (ratio of 2.3:1.0) of 1,1-bis-(2-propynyl)hydrazine (**4**) and 1-(2-propynyl)hydrazine (**5**) (0.24 g) in methanol (2 mL), were stirred at 25 °C for 2 h. Removal of the solvent in vacuo at 25 °C afforded an oil which was separated by silica gel flash column chromatography. Petroleum ether (boiling point range of 40–60 °C) was used for all chromatographic separations. Elution with petroleum ether–ethyl acetate (98:2, v/v to 96:4, v/v ratio) gave the respective bis-(2-propynyl)hydrazine product [**(*E*)-7**]. Continued elution with petroleum ether–ethyl acetate (97:3, v/v to 88:12, v/v ratio) yielded a mixture of the respective (*E*)- and (*Z*)-mono-(2-propynyl)hydrazine stereoisomers [**(*E*)-8** and **(*Z*)-8**]. Products [**(*E*)-7**, and the mixture of **(*E*)-8** and **(*Z*)-8**] were stored at −78 °C prior to their use in biological studies. The % chemical yield, (*E*):(*Z*) ratio of compounds **8** at 25 °C as determined from the ^{1}H NMR integrals for the C*H*$_2$ resonances of the two isomers, and spectral data (IR, ^{1}H NMR, ^{13}C NMR) for compounds **(*E*)-7**, and **(*E*)-8** and **(*Z*)-8**, are listed below.

5.3.1. (*E*)-*N*-Bis-(2-propynyl)-2-(2-methylphenyl)ethyidene]-hydrazine [(*E*)-7a], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(2-methylphenyl)ethylidene]hydrazines [(*E*)-8a and (*Z*)-8a]

(E)-7a: Yellow oil separated using petroleum ether–ethyl acetate as eluant (96:4, v/v); yield, 15%; IR (liquid film): 3294 (C≡CH), 1602 (CH=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ *7.14–7.24* (m, 4H, phenyl hydrogens), 7.00 (t, *J* = 5.7 Hz, 1H, CH_2CH=N), 3.96 (d, *J* = 2.4 Hz, 4H, CH_2C≡CH), 3.62 (d, *J* = 5.7 Hz, 2H, CH_2CH=N), 2.34 (s, 3H, CH_3) 2.26 (t, *J* = 2.4 Hz, 2H, CH_2C≡*CH*); ^{13}C NMR ($CDCl_3$): δ 141.78 (CH=N), 136.81, 135.84 (phenyl C-1, C-2), 130.24, 129.26 (phenyl C-3, C-6), 126.67, 125.99 (phenyl C-4, C-5), 73.41 (*C*≡CH), 61.30 (C≡CH), 42.23 (CH_2C≡CH), 37.38 (CH_2CH=N), 19.60 (CH_3).

(E)-8a and (Z)-8a (ratio 3.5:1): Yellow oil separated using petroleum ether–ethyl acetate (88:12, v/v); yield, 21%; IR (liquid film): 3294 (C≡CH), 1602 (CH=N) cm^{-1}; **(*E*)-8a isomer:** ^{1}H NMR ($CDCl_3$): δ 7.17–7.20 (m, 4H, phenyl hydrogens), 7.06 (t, *J* = 5.7 Hz, 1H, CH_2CH=N), 5.20 (br s, 1H, N*H*), 3.90 (d, *J* = 2.4 Hz, 2 H, CH_2C≡CH), 3.57 (d, *J* = 5.7 Hz, 2H, CH_2CH=N), 2.34 (s, 3H, CH_3), 2.25 (t, *J* = 2.4 Hz, 1H, CH_2C≡*CH*); ^{13}C NMR ($CDCl_3$): δ 142.15 (*C*H=N), 136.76, 135.58 (phenyl C-1, C-2), 130.30, 129.34 (phenyl C-3, C-6), 126.76, 126.06 (phenyl C-4, C-5), 72.09 (*C*≡CH), 61.00 (C≡*C*H), 39.05 (CH_2C≡CH), 36.81 (CH_2CH=N), 19.44 (CH_3); *(Z)-8a isomer:* ^{1}H NMR ($CDCl_3$): δ 7.17–7.20 (m, 4H, phenyl hydrogens), 6.70 (t, *J* = 4.9 Hz, 1H, CH_2CH=N), 5.20 (br s, 1H, N*H*), 4.00 (d, *J* = 2.1 Hz, 2H, CH_2C≡CH), 3.42 (d, *J* = 4.9 Hz, 2H, CH_2CH=N), 2.36 (s, 3H, CH_3), 2.31 (t, *J* = 2.1 Hz, 1H, CH_2C≡*CH*); ^{13}C NMR ($CDCl_3$): δ 142.85 (*C*H=N), 136.76, 135.58 (phenyl C-1, C-2), 130.46, 129.05 (phenyl C-3, C-6), 127.08, 125.99 (phenyl C-4, C-5), 71.80 (*C*≡CH), 61.00 (C≡CH), 40.45 (CH_2C≡CH), 31.42 (CH_2CH=N), 19.44 (CH_3).

5.3.2. (*E*)-*N*-Bis-(2-propynyl)-2-(2-methoxyphenyl)-ethylidene]hydrazine [(*E*)-7b], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(2-methoxyphenyl)ethylidene]hydrazines [(*E*)-8b and (*Z*)-8b]

***(E)*-7b**: Yellow oil separated using petroleum ether–ethyl acetate (96:4, v/v) as eluant; yield: 12%; IR (liquid film): 3274 (C≡CH), 1716 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.20–7.26 (m, 2H, phenyl H-4, H-6), 7.10 (t, J = 5.4 Hz, 1H, CH_2CH=N), 6.86–6.93 (m, 2H, phenyl H-3, H-5), 3.95 (d, J = 2.4 Hz, 4H, CH_2C≡CH), 3.84 (s, 3H, OCH_3), 3.62 (d, J = 5.4 Hz, 2H, CH_2CH=N), 2.25 (t, J = 2.4 Hz, 2H, CH_2C≡CH); ^{13}C NMR ($CDCl_3$): δ 157.35 (phenyl C-2), 143.18 (*C*H=N), 130.14 (phenyl C-6), 127.74 (phenyl C-4), 126.24 (phenyl C-1), 120.51 (phenyl C-5), 110.37 (phenyl C-3), 73.27 (*C*≡CH), 61.00 (C≡*C*H), 55.36 (OCH_3), 42.29 (CH_2C≡CH), 33.80 (*C*H_2CH=N).

(*E*)-8b and (*Z*)-8b (ratio 1.6:1): Yellow oil separated using petroleum ether–ethyl acetate (89:11, v/v); yield: 27%; IR (liquid film): 3271 (C≡CH), 1595 (CH=N) cm^{-1}; **(*E*)-8b isomer:** ^1H NMR ($CDCl_3$): δ 7.13–7.28 (m, 3H, phenyl H-4, H-6, C*H*=N), 6.85–6.95 (m, 2H, phenyl H-3, H-5), 4.91 (br s, 1H, N*H*), 3.88 (d, J = 2.4 Hz, 2H, CH_2 C≡CH), 3.84 (s, 3H, OCH_3), 3.57 (d, J = 5.4 Hz, 2H, CH_2 CH=N), 2.25 (t, J = 2.4 Hz, 1H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 157.32 (phenyl C-2), 143.05 (*C*H=N), 130.24 (phenyl C-6), 127.85 (phenyl C-4), 125.94 (phenyl C-1), 120.59 (phenyl C-5), 110.37 (phenyl C-3), 72.02 (*C*≡CH), 61.00 (C≡*C*H), 55.36 (OCH_3), 39.14 (*C*H_2C≡CH), 33.42 (*C*H_2CH=N); **(*Z*)-8b isomer:** ^1H NMR ($CDCl_3$): δ 7.13–7.28 (m, 2H, phenyl H-4, H-6), 6.85–6.95 (m, 2H, phenyl H-3, H-5), 6.72 (t, J = 5.1 Hz, 1H, CH_2CH=N), 5.30 (br s, 1H, N*H*), 3.98 (d, J = 2.1 Hz, 2H, CH_2 C≡CH), 3.86 (s, 3H, OCH_3), 3.44 (d, J = 5.1 Hz, 2H, CH_2CH=N), 2.28 (t, J = 2.1 Hz, 1H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 157.32 (phenyl C-2), 143.33 (*C*H=N), 130.20 (phenyl C-6), 128.16 (phenyl C-4), 125.94 (phenyl C-1), 120.79 (phenyl C-5), 110.44 (phenyl C-3), 71.62 (*C*≡CH), 61.00 (C≡*C*H), 55.36 (OCH_3), 40.45 (*C*H_2C≡CH), 28.41 (*C*H_2CH=N).

5.3.3. (*E*)- and (*Z*)-*N*-(2-Propynyl)-2-(2-chlorophenyl)-ethylidene]hydrazines [(*E*)-8c and (*Z*)-8c, ratio 3.1:1]

Yellow oil isolated using petroleum ether–ethyl acetate (96:4, v/v) as eluant; yield, 34%; IR (liquid film): 3278 (C≡CH), 1602 (CH=N) cm^{-1}; **(*E*)-8c isomer:** ^1H NMR ($CDCl_3$): δ 7.18–7.42 (m, 4H, phenyl hydrogens), 7.14 (t, J = 5.7 Hz, 1H, CH_2CH=N), 5.10 (br s, 1H, N*H*), 3.89 (d, J = 2.4 Hz, 2H, CH_2C≡CH), 3.70 (d, J = 5.7 Hz, 2H, CH_2CH=N), 2.26 (t, J = 2.4 Hz, 1H, CH_2CCH); ^{13}C NMR ($CDCl_3$): δ 140.72 (*C*H=N), 135.44 (phenyl C-1), 134.07 (phenyl C-2), 130.78 (phenyl C-6), 129.60 (phenyl C-3), 127.90 (phenyl C-4), 126.93 (phenyl C-5), 72.18 (*C*≡CH), 61.10 (C≡*C*H), 38.98 (*C*H_2C≡CH), 36.62 (*C*H_2CH=N); **(*Z*)-8c isomer:** ^1H NMR ($CDCl_3$): δ 7.18–7.42 (m, 4H, phenyl hydrogens), 6.71 (t, J = 5.1 Hz, 1H, CH_2CH=N), 5.10 (br s, 1H, N*H*), 4.00 (d, J = 2.1 Hz, 2H, CH_2C≡CH), 3.56 (d, J = 5.1 Hz, 2H, CH_2CH=N), 2.30 (t, J = 2.1 Hz, 1H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 140.59 (*C*H=N), 135.44 (phenyl C-1), 134.07 (phenyl C-2), 131.21 (phenyl C-6), 129.70 (phenyl C-3), 128.40 (phenyl C-4), 126.76 (phenyl C-5), 71.86 (*C*≡CH), 61.10 (C≡*C*H), 40.50 (*C*H_2C≡CH), 31.38 (*C*H_2CH=N).

5.3.4. (*E*)-*N*-Bis-(2-propynyl)-2-(2-fluorophenyl)ethyidene]-hydrazine [(*E*)-7d], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(2-fluorophenyl)ethylidene]hydrazines [(*E*)-8d and (*Z*)-8d]

(*E*)-7d: Yellow oil separated using petroleum ether–ethyl acetate (96:4, v/v) as eluant; yield, 18%; IR (liquid film): 3300 (C≡CH), 1710 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ *7.05–7.29* (m, 4H, phenyl hydrogents), 7.03 (t, J = 5.7 Hz, 1H, CH_2CH=N), 3.97 (d, J = 2.4 Hz, 4H, CH_2C≡CH), 3.65 (d, J = 5.7 Hz, 2H, *C*H_2CH=N), 2.26 (t, J = 2.4 Hz, 2H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 162.45 (d, $^1J_{C,F}$ = 246.1 Hz, C-2), 140.29 (*C*H=N), 130.85 (d, $^3J_{C,F}$ = 4.37 Hz, C-6), 128.25 (d, $^3J_{C,F}$ = 8.75 Hz, C-4), 124.76 (d, $^2J_{C,F}$ = 15.39 Hz, C-1), 124.07 (d, $^4J_{C,F}$ = 3.3 Hz, C-5), 115.31 (d, $^2J_{C,F}$ = 21.96 Hz, C-3), 73.41 (*C*≡CH), 61.30 (C≡*C*H), 42.20 (*C*H_2C≡CH), 32.63 (*C*H_2CH=N).

(*E*)-8d and (*Z*)-8d (ratio 3.7:1): Yellow oil separated using petroleum ether–ethyl acetate (94:6, v/v) as eluant; yield, 24%; IR (liquid film): 3288 (C≡CH), 1649 (CH=N) cm^{-1}; **(*E*)-8d isomer:** ^1H NMR ($CDCl_3$): δ 7.01–7.28 (m, 5H, phenyl hydrogens, CH_2CH=N), 5.10 (br s, 1H, N*H*), 3.90 (d, J = 2.4 Hz, 2H, CH_2C≡CH), 3.61 (d, J = 6.0 Hz, 2H, CH_2CH=N), 2.26 (t, J = 2.4 Hz, 1H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 162.45 (d, $^1J_{C,F}$ = 246.1 Hz, C-2), 140.81 (*C*H=N), 130.78 (d, $^3J_{C,F}$ = 4.37 Hz, C-6), 128.30 (d, $^3J_{C,F}$ = 8.75 Hz, C-4), 124.50 (d, $^2J_{C,F}$ = 15.40 Hz, C-1), 124.03 (d, $^4J_{C,F}$ = 3.24 Hz, C-5), 115.44 (d, $^2J_{C,F}$ = 21.56 Hz, C-3), 72.11 (*C*≡CH), 61.30 (C≡*C*H), 38.88 (*C*H_2C≡CH), 32.08 (*C*H_2CH=N); **(*Z*)-8d isomer:** ^1H NMR ($CDCl_3$): δ 7.01–7.28 (m, 4H, phenyl hydrogens), 6.75 (t, J = 5.1 Hz, 1H, CH_2CH=N), 5.10 (br s, 1H, N*H*), 4.00 (d, J = 2.1 Hz, 2H, CH_2C≡CH), 3.48 (d, J = 5.1 Hz, 2H, CH_2CH=N), 2.30 (t, J = 2.1 Hz, 1H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 162.45 (d, $^1J_{C,F}$ = 246.1 Hz, C-2), 141.50 (*C*H=N), 130.44 (d, $^3J_{C,F}$ = 3.32 Hz, C-6), 128.61 (d, $^3J_{C,F}$ = 7.69 Hz, C-4), 124.50 (d, $^2J_{C,F}$ = 15.40 Hz, C-1), 124.07 (d, $^4J_{C,F}$ = 3.24 Hz, C-5), 115.20 (d, $^2J_{C,F}$ = 17.58 Hz, C-3), 71.80 (*C*≡CH), 61.30 (C≡*C*H), 40.40 (*C*H_2C≡CH), 26.55 (*C*H_2CH=N).

5.3.5. (*E*)-*N*-Bis-(2-propynyl)-2-(2-trifluoromethylphenyl)-ethyidene]hydrazine [(*E*)-7e], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(2-trifluoromethylphenyl)ethylidene]hydrazines [(*E*)-8e and (*Z*)-8e]

(*E*)-7e: Yellow oil separated using petroleum ether–ethyl acetate (96:4, v/v) as eluant; yield, 17%; IR (liquid film): 3288 (C≡CH), 1736 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.31–7.66 (m, 4H, phenyl hydrogens), 7.00 (t, J = 5.7 Hz, 1H, CH_2CH=N), 3.98 (d, J = 2.4 Hz, 4H, CH_2C≡CH), 3.80 (d, J = 5.7 Hz, 2H, CH_2CH=N), 2.26 (t, J = 2.4 Hz, 2H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 140.46 (*C*H=N), 136.10 (phenyl C-1), 131.81, 131.46 (phenyl C-5, C-6), 126.57 (phenyl C-4), 126.22 (q, $^1J_{C,F}$ = 270.2 Hz, *C*F_3), 125.93 (d, $^3J_{C,F}$ 8 5.50 Hz, phenyl C-3), 121.00 (q, $^2J_{C,F}$ = 29.66 Hz, phenyl C-2), 73.47 (*C*≡CH), 61.30 (C≡*C*H), 42.17 (*C*H_2C≡CH), 36.10 (*C*H_2CH=N).

(*E*)-8e and (*Z*)-8e (ratio 4.3:1): Yellow oil separated using petroleum ether–ethyl acetate (92:8, v/v) as eluant; yield, 23%; IR (liquid film): 3288 (C≡CH), 1750 (CH=N) cm^{-1}; **(*E*)-8e isomer:** ^1H NMR ($CDCl_3$): δ *7.31–7.66* (m, 4H, phenyl hydrogens), 7.09 (t, J = 5.4 Hz, 1H, CH_2CH=N), 5.10 (br s, 1H, N*H*), 3.90 (d, J = 2.1 Hz, 2H, CH_2C≡CH), 3.75 (d, J = 5.4 Hz, 2H, CH_2CH=N), 2.26 (t, J = 2.1 Hz, 1H, CH_2C≡C*H*); **(*Z*)-8e isomer:** ^1H NMR ($CDCl_3$): δ *7.31–7.66* (m, 4H, phenyl hydrogens), 6.72 (t, J = 5.1 Hz, 1H, CH_2CH=N), 5.10 (br s, 1H, N*H*), 4.00 (d, J = 1.8 Hz, 2H, CH_2C≡CH), 3.62 (d, J = 5.1 Hz, 2H, CH_2CH=N), 2.31 (t, J = 1.8 Hz, 1H, CH_2C≡C*H*).

5.3.6. (*E*)-*N*-Bis-(2-propynyl)-2-(3-methylphenyl)ethyidene]-hydrazine [(*E*)-7f], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(3-methylphenyl)ethylidene]hydrazines [(*E*)-8f and (*Z*)-8f]

***(E)*-7f**: Yellow oil separated using petroleum ether–ethyl acetate as eluant (98:2, v/v); yield, 14%; IR (liquid film): 3301 (C≡CH), 1743 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.21 (t, J = 7.3 Hz, 1H, phenyl H-5), 7.02–7.08 (m, 4H, phenyl H-2, H-4, H-6, CH_2CH=N), 3.98 (d, J = 2.4 Hz, 4H, CH_2C≡CH), 3.58 (d, J = 5.4 Hz, 2H, CH_2CH=N), 2.33 (s, 3H, CH_3), 2.26 (t, J = 2.4 Hz, 2H, CH_2C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 142.44 (*C*H=N), 138.13, 137.55 (phenyl C-1, C-3), 129.64, 128.40 (phenyl C-2, C-5), 127.21 (phenyl C-4), 125.89 (phenyl C-6), 73.41 (*C*≡CH), 61.30 (C≡*C*H), 42.26 (*C*H_2C≡CH), 39.40 (*C*H_2CH=N).

(*E*)-8f and (*Z*)-8f (ratio 2.9:1): Yellow oil separated using petroleum ether–ethyl acetate (95:5, v/v) as eluant; yield, 26%; IR (liquid film): 3301 (C≡CH), 1595 (CH=N) cm^{-1}; **(*E*)-8f isomer:** ^1H NMR ($CDCl_3$): δ 7.21 (t, J = 6.0 Hz, 1H, CH_2CH=N), 7.05–7.13 (m, 4H, phenyl hydrogens), 5.08 (br s, 1 H, N*H*), 3.91 (d, J = 2.7 Hz, 2H, CH_2C≡CH), 3.54 (d, J = 6.0 Hz, 2H, CH_2CH=N), 2.34 (s, 3H, CH_3),

2.28 (t, J = 2.7 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 142.85 (*C*H=N), 138.22, 137.24 (phenyl C-1, C-3), 129.61, 128.46 (phenyl C-2, C-5), 127.86 (phenyl C-4), 125.86 (phenyl C-6), 72.13 (*C*≡CH), 60.30 (C≡*C*H), 39.10 (*C*H$_2$C≡CH), 38.91 (*C*H$_2$CH=N), 21.36 (*C*H$_3$); **(*Z*)-8f isomer:** ^1H NMR ($CDCl_3$): δ 7.05–7.13 (m, 4H, phenyl hydrogens), 6.81 (t, J = 5.4 Hz, 1H, CH$_2$C*H*=N), 5.08 (br s, 1H, N*H*), 3.99 (d, J = 2.4 Hz, 2H, C*H*$_2$C≡CH), 3.43 (d, J = 5.4 Hz, 2H, C*H*$_2$CH=N), 2.36 (s, 3H, C*H*$_3$), 2.30 (t, J = 2.4 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 142.85 (*C*H=N), 138.22, 137.24 (phenyl C-1, C-3), 129.32, 128.73 (phenyl C-2, C-5), 127.28 (phenyl C-4), 125.59 (phenyl C-6), 72.13 (*C*≡CH), 60.30 (C≡*C*H), 40.45 (*C*H$_2$C≡CH), 33.22 (*C*H$_2$CH=N), 21.36 (*C*H$_3$).

5.3.7. (*E*)-*N*-Bis-(2-propynyl)-2-(3-methoxyphenyl)ethyidene]-hydrazine [(*E*)-7g], and (*E*)- and (*Z*)-*N*-(2-Propynyl)-2-(3-methoxyphenyl)ethylidene]hydrazines [(*E*)-8g and (*Z*)-8g]

(*E*)-7g: Yellow oil separated using petroleum ether–ethyl acetate as eluant (95:5, v/v); yield, 12%; IR (liquid film): 3301 (C≡CH), 1709 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.22 (t, J = 7.3 Hz, 1H, phenyl H-5), 7.04 (t, J = 5.7 Hz, 1H, CH$_2$C*H*=N), 6.76–6.84 (m, 3H, phenyl H-2, H-4, H-6), 3.97 (d, J = 2.4 Hz, 4H, C*H*$_2$C≡CH), 3.80 (s, 3H, OC*H*$_3$), 3.59 (d, J = 5.7 Hz, 2H, C*H*$_2$CH=N), 2.26 (t, J = 2.4 Hz, 2H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 157.21 (phenyl C-3), 141.99 (*C*H=N), 139.22 (phenyl C-1), 129.45 (phenyl C-5), 121.21 (phenyl C-6), 114.49 (phenyl C-2), 112.04 (phenyl C-5), 73.47 (*C*≡CH), 61.30 (C≡*C*H), 55.21 (OCH$_3$), 42.23 (*C*H$_2$C≡CH), 39.56 (*C*H$_2$CH=N).

(*E*)-8g and (*Z*)-8g (ratio 3:1): Yellow oil separated using petroleum ether–ethyl acetate (93:7 v/v) as eluant; yield, 18%; IR (liquid film): 3301 (C≡CH), 1595 (CH=N) cm^{-1}; **(*E*)-8g isomer:** ^1H NMR ($CDCl_3$): δ 7.25 (t, J = 7.3 Hz, 1H, phenyl H-5), 7.16 (t, J = 6.0 Hz, 1H, C*H*=N), 6.77–6.84 (m, 3H, phenyl H-2, H-4, H-6), 5.08 (br s, 1H, N*H*), 3.90 (d, J = 2.7 Hz, 2H, C*H*$_2$C≡CH), 3.80 (s, 3H,OC*H*$_3$), 3.55 (d, J = 6.0 Hz, 2H, C*H*$_2$CH=N), 2.27 (t, J 8 2.7 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 159.78 (phenyl C-3), 142.44 (*C*H=N), 138.92 (phenyl C-1), 129.51 (phenyl C-5), 121.15 (phenyl C-6), 114.46 (phenyl C-2), 112.08 (phenyl C-4), 72.15 (*C*≡CH), 61.30 (C≡*C*H), 55.17 (OCH$_3$) 39.02 (*C*H$_2$C≡CH), 39.00 (*C*H$_2$CH=N); **(*Z*)-8g isomer:** ^1H NMR ($CDCl_3$): δ 7.25 (t, J = 7.3 Hz, 1H, phenyl H-5), 6.70–6.84 (m, 4H, phenyl H-2, H-4, H-6, C*H*=N), 5.08 (br s, 1H, N*H*), 3.99 (d, J = 2.4 Hz, 2H, C*H*$_2$C≡CH), 3.81 (s, 3H, OC*H*$_3$), 3.44 (d, J = 4.8 Hz, 2H, C*H*$_2$CH=N), 2.30 (t, J = 2.4 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 159.78 (phenyl C-3), 142.99 (*C*H=N), 138.92 (phenyl C-1), 129.69 (phenyl C-5), 120.88 (phenyl C-6), 114.27 (phenyl C-2), 112.23 (phenyl C-4), 71.81 (*C*≡CH), 61.30 (C≡*C*H), 55.17 (OCH$_3$) 40.42 (*C*H$_2$C≡CH), 33.32 (*C*H$_2$CH=N).

5.3.8. (*E*)-*N*-Bis-(2-propynyl)-2-(3-chlorophenyl)ethyidene]-hydrazine [(*E*)-7h], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(3-chlorophenyl)ethylidene]hydrazines [(*E*)-8h and (*Z*)-8h]

(*E*)-7h: Yellow oil separated using petroleum ether–ethyl acetate as eluant (97:3, v/v); yield, 12%; IR (liquid film): 3301 (C≡CH), 1650 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ *7.08–7.32* (m, 4H, phenyl hydrogens), 7.00 (t, J = 5.7 Hz, 1H, CH$_2$C*H*=N), 3.99 (d, J = 2.4 Hz, 4H, C*H*$_2$C≡CH), 3.59 (d, J = 5.7 Hz, 2H, C*H*$_2$CH=N), 2.28 (t, J = 2.4 Hz, 2H, CH$_2$CC*H*); ^{13}C NMR ($CDCl_3$): δ 141.23 (*C*H=N), 139.44 (phenyl C-1), 134.35 (phenyl C-3), 129.73 (phenyl C-5), 128.93 (phenyl C-2), 126.93, 126.73 (phenyl C-4, C-6), 72.30 (*C*≡CH), 61.00 (C≡*C*H), 42.26 (*C*H$_2$C≡CH), 38.54 (*C*H$_2$CH=N).

(*E*)-8h and (*Z*)-8h (ratio 4.3:1): Yellow oil separated using petroleum ether–ethyl acetate (93:7 v/v) as eluant; yield, 18%; IR (liquid film): 3288 (C≡CH), 1689 (CH=N) cm^{-1}; **(*E*)-8h isomer:** ^1H NMR ($CDCl_3$): δ *7.10–7.31* (m, 5H, phenyl hydrogens, CH$_2$C*H*=N), 5.10 (br s, 1H, N*H*), 3.95 (d, J = 2.1 Hz, 2H, C*H*$_2$C≡CH), 3.58 (d, J = 6.0 Hz, 2H, C*H*$_2$CH=N), 2.33 (t, J = 2.1 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 141.23 (*C*H=N), 139.44 (phenyl C-1), 134.35 (phenyl C-3), 129.73 (phenyl C-5), 128.93 (phenyl C-2), 126.99, 126.72 (phenyl C-4, C-6), 72.29 (*C*≡CH), 61.00 (C≡*C*H), 38.92 (*C*H$_2$C≡CH), 38.54 (*C*H$_2$CH=N); **(*Z*)-8h isomer:** ^1H NMR ($CDCl_3$): δ *7.10–7.31* (m, 4H, phenyl hydrogens), 6.80 (t, J = 5.1 Hz, 1H, CH$_2$C*H*=N), 5.10 (br s, 1H, N*H*), 4.03 (d, J = 1.8 Hz, 2H, C*H*$_2$C≡CH), 3.48 (d, J = 5.1 Hz, 2H, C*H*$_2$CH=N), 2.35 (t, J = 1.8 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 141.23 (*C*H=N), 139.44 (phenyl C-1), 134.35 (phenyl C-3), 130.04 (phenyl C-5), 128.70 (phenyl C-2), 126.99, 126.72 (phenyl C-4, C-6), 71.87 (C≡CH), 61.00 (C≡*C*H), 40.44 (*C*H$_2$C≡CH), 32.81 (*C*H$_2$CH=N).

5.3.9. (*E*)- and (*Z*)-*N*-(2-Propynyl)-2-(3-fluorophenyl)ethylidene]hydrazines [(*E*)-8i and (*Z*)-8i, ratio 5.2:1]

Yellow oil separated using petroleum ether–ethyl acetate (93:7, v/v) as eluant; yield, 22%; IR (liquid film): 3294 (C≡CH), 1703 (CH=N) cm^{-1}; **(*E*)-8i isomer:** ^1H NMR ($CDCl_3$): δ *7.21–7.31* (m, 1H, phenyl H-5), 7.09 (t, J = 6.0 Hz, 1H, CH$_2$C*H*=N), 6.90–7.14 (m, 3H, phenyl H-2, H-4, H-6), 5.10 (br s, 1H, N*H*), 3.91 (d, J = 2.4 Hz, 2H, C*H*$_2$C≡CH), 3.56 (d, J = 6.0 Hz, 2H, C*H*$_2$CH=N), 2.28 (t, J = 2.4 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 164.54 (d, $^1J_{C,F}$ = 246.1 Hz, phenyl C-3), 141.38 (*C*H=N), 140.01 (phenyl C-1), 129.94 (d, $^3J_{C,F}$ = 8.75 Hz, phenyl C-5), 124.43 (d, $^4J_{C,F}$ = 2.19 Hz, phenyl C-6), 115.73 (d, $^2J_{C,F}$ = 22.0 Hz, phenyl C-2), 113.45 (d, $^2J_{C,F}$ = 20.90 Hz, phenyl C-4), 72.25 (*C*≡CH), 60.10 (C≡*C*H), 38.95 (*C*H$_2$C≡CH), 38.62 (*C*H$_2$CH=N); **(*Z*)-8i isomer:** ^1H NMR ($CDCl_3$): δ *7.21–7.31* (m, 1H, phenyl H-5), 6.90–7.14 (m, 3H, phenyl H-2, H-4, H-6), 6.78 (t, J = 4.8 Hz, 1H, CH$_2$C*H*=N), 5.10 (br s, 1H, N*H*), 3.98 (d, J = 2.1 Hz, 2H, C*H*$_2$C≡CH), 3.46 (d, J = 4.8 Hz, 2H, C*H*$_2$CH=N), 2.30 (t, J = 2.1 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 164.54 (d, $^1J_{C,F}$ = 246.1 Hz, phenyl C-3), 141.95 (*C*H=N), 140.01 (phenyl C-1), 130.30 (d, $^3J_{C,F}$ = 8.75 Hz, phenyl C-5), 124.22 (d, $^4J_{C,F}$ = 3.30 Hz, phenyl C-6), 115.75 (d, $^2J_{C,F}$ = 21.96 Hz, phenyl C-2), 113.78 (d, $^2J_{C,F}$ = 20.90 Hz, phenyl C-4), 71.91 (*C*≡CH), 60.10 (C≡*C*H), 40.45 (*C*H$_2$C≡CH), 32.94 (*C*H$_2$CH=N).

5.3.10. (*E*)-*N*-Bis-(2-propynyl)-2-(3-trifluoromethylphenyl)-ethyidene]hydrazine [(*E*)-7j], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(3-trifluoromethylphenyl)ethylidene]hydrazines [(*E*)-8j and (*Z*)-8j]

(*E*)-7j: Yellow oil separated using petroleum ether–ethyl acetate as eluant (96:4, v/v); yield, 14%; IR (liquid film): 3294 (C≡CH), 1736 (CH=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ *7.32–7.54* (m, 4H, phenyl hydrogens), 7.02 (t, J = 5.7 Hz, 1H, CH$_2$C*H*=N), 3.99 (d, J = 2.4 Hz, 4H, C*H*$_2$C≡CH), 3.68 (d, J = 5.7 Hz, 4H, C*H*$_2$CH=N), 2.27 (t, J = 2.4 Hz, 2H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 140.11 (*C*H=N), 138.73 (phenyl C-1), 132.60 (phenyl C-6), 130.56 (q, $^2J_{C,F}$ = 29.66 Hz, phenyl C-3), 128.84 (phenyl C-5), 125.57 (d, $^3J_{C,F}$ = 3.24 Hz, phenyl C-2), 124.09 (q, $^1J_{C,F}$ = 272.46 Hz, *C*F$_3$), 123.32 (d, $^3J_{C,F}$ = 3.32 Hz, phenyl C-4), 73.62 (*C*≡CH), 61.50 (C≡*C*H), 42.15 (*C*H$_2$C≡CH), 39.20 (*C*H$_2$CH=N).

(*E*)-8j and (*Z*)-8j (ratio 6:1): Yellow oil separated using petroleum ether–ethyl acetate (90:10, v/v) as eluant; yield, 21%; IR (liquid film): 3301 (C≡CH), 1736 (CH=N) cm^{-1}; **(*E*)-8j isomer:** ^1H NMR ($CDCl_3$): δ 7.39–7.52 (m, 4H, phenyl hydrogens), 7.21 (t, J = 5.4 Hz, 1H, CH$_2$C*H*=N), 5.12 (br s, 1H, N*H*), 3.92 (d, J = 2.7 Hz, 2H, C*H*$_2$C≡CH), 3.63 (d, J = 5.4 Hz, 2H, C*H*$_2$CH=N), 2.28 (t, J = 2.7 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 140.11 (*C*H=N), 138.73 (phenyl C-1), 132.60 (phenyl C-6), 130.56 (q, $^2J_{C,F}$ = 29.66 Hz, phenyl C-3), 128.86 (phenyl C-5), 125.57 (d, $^3J_{C,F}$ = 3.24 Hz, C_2 aryl), 124.08 (q, $^1J_{C,F}$ = 272.46 Hz, *C*F$_3$), 123.32 (d, $^3J_{C,F}$ = 3.32 Hz, phenyl C-4), 73.62 (*C*≡CH), 61.50 (C≡*C*H), 42.15 (*C*H$_2$C≡CH), 39.20 (*C*H$_2$CH=N); **(*Z*)-8j isomer:** ^1H NMR ($CDCl_3$): δ *7.39–7.52* (m, 4H, phenyl hydrogens), 6.77 (t, J = 5.1 Hz, 1H, CH$_2$C*H*=N), 5.12 (br s, 1H, N*H*), 4.00 (d, J = 2.4 Hz, 2H, CH$_2$C≡CH), 3.52 (d, J = 5.1 Hz, 2H, C*H*$_2$CH=N), 2.30 (t, J = 2.4 Hz, 1H, CH$_2$C≡C*H*); ^{13}C NMR ($CDCl_3$): δ 140.11 (*C*H=N), 138.73 (phenyl C-1), 132.30 (phenyl C-6), 130.56 (q, $^2J_{C,F}$ = 29.66 Hz, phenyl

C-3), 129.26 (phenyl C-5), 125.37 (phenyl C-2), 124.08 (q, $^{1}J_{C,F}$ = 272.46 Hz, CF_3), 123.54 (d, $^{3}J_{C,F}$ = 3.24 Hz, phenyl C-4), 73.62 ($C{\equiv}CH$), 61.50 ($C{\equiv}CH$), 40.45 ($CH_2C{\equiv}CH$), 33.60 ($CH_2CH{=}N$).

5.3.11. (*E*)-*N*-Bis-(2-propynyl)-2-(4-methylphenyl)ethyidene]-hydrazine [(*E*)-7k], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(4-methylphenyl)ethylidene]hydrazines [(*E*)-8k and (*Z*)-8k]

(*E*)-7k: Yellow oil separated using petroleum ether–ethyl acetate as eluant (98:2, v/v); yield, 18%; IR (liquid film): 3287 (C≡CH), 1517 (CH=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ *7.10–7.18* (m, 4H, phenyl hydrogens), 7.03 (t, *J* = 5.7 Hz, 1H, $CH_2CH{=}N$), 3.97 (d, *J* = 2.4 Hz, 4H, $CH_2C{\equiv}CH$), 3.58 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.33 (s, 3H, CH_3), 2.26 (t, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 142.59 (*C*H=N), 136.02 (phenyl C-4), 134.48 (phenyl C-1), 129.28, 129.19 (phenyl C-2, C-6, C3-, C-5), 73.43 (*C*≡CH), 61.26 (C≡CH), 42.21 ($CH_2C{\equiv}CH$), 39.11 ($CH_2CH{=}N$), 21.04 (CH_3).

(*E*)-8k and (*Z*)-8k (ratio 3.4:1): Yellow oil separated using petroleum ether–ethyl acetate (90:10 v/v) as eluant; yield, 21%; IR (liquid film): 3301 (C≡CH), 1703 (CH=N) cm^{-1}; **(*E*)-8k isomer:** ^{1}H NMR ($CDCl_3$): δ 7.09–7.26 (m, 5H, phenyl hydrogens, $CH_2CH{=}N$), 5.06 (br s, 1H, N*H*), 3.91 (d, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$), 3.53 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.33 (s, 3H, CH_3), 2.27 (t, *J* = 2.4 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 142.89 (*C*H=N), 136.09 (phenyl C-4), 134.27 (phenyl C-1), 129.25 (phenyl C-2, C-6), 128.73 (phenyl C-3, C-5), 72.83 (*C*≡CH), 61.26 (C≡CH), 39.07 ($CH_2C{\equiv}CH$), 38.92 ($CH_2CH{=}N$), 21.03 (CH_3); **(*Z*)-8k isomer:** ^{1}H NMR ($CDCl_3$): δ 7.09–7.26 (m, 4H, phenyl hydrogens), 6.80 (t, *J* = 5.4 Hz, 1H, CH_2CHN), 5.06 (br s, 1H, N*H*), 3.98 (d, *J* = 2.1 Hz, 2H, $CH_2C{\equiv}CH$), 3.43 (d, *J* = 5.4 Hz, 2H, $CH_2CH{=}N$), 2.34 (s, 3H, CH_3), 2.30 (t, *J* = 2.1 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 142.89 (*C*H=N), 136.09 (phenyl C-4), 134.27 (phenyl C-1), 129.51 (phenyl C-2, C-6), 128.45 (phenyl C-3, C-5), 72.12 (*C*≡CH), 61.26 (C≡CH), 40.45 ($CH_2C{\equiv}CH$), 32.87 ($CH_2CH{=}N$), 21.03 (CH_3).

5.3.12. (*E*)-*N*-Bis-(2-propynyl)-2-(4-methoxyphenyl)ethyidene]-hydrazine [(*E*)-7l], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(4-methoxyphenyl)ethylidene]hydrazines [(*E*)-8l and (*Z*)-8l]

(*E*)-7l: Yellow oil separated using petroleum ether–ethyl acetate as eluant (94:6, v/v); yield, 15%; IR (liquid film): 3287 (C≡CH), 1607 (CH=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.11–7.15 (d, *J* = 8.7 Hz, 2 H, phenyl H-2, H-6), 7.03 (t, *J* = 5.7 Hz, 1H, $CH_2CH{=}N$), 6.84–6.89 (d, *J* = 8.7 Hz, 2H, phenyl H-3, H-5), 3.96 (d, *J* = 2.4 Hz, 4 H, $CH_2C{\equiv}CH$), 3.80 (s, 3H, OCH_3), 3.56 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.26 (t, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 158.28 (phenyl C-4) 142.70 (*C*H=N), 129.82 (phenyl C-2, C-6), 129.58 (phenyl C-1), 113.96 (phenyl C-3, C-5), 73.44 (*C*≡CH), 61.10 (C≡CH), 55.29 (OCH_3), 42.23 ($CH_2C{\equiv}CH$), 38.66 ($CH_2CH{=}N$).

(*E*)-8l and (*Z*)-8l (ratio 3.3:1): Yellow oil separated using petroleum ether–ethyl acetate (93:7, v/v) as eluant; yield, 25%; IR (liquid film): 3287 (C≡CH), 1607 (CH=N) cm^{-1}; **(*E*)-8l isomer:** ^{1}H NMR ($CDCl_3$): δ 7.15–7.19 (m, 2H, phenyl H-2, H-6), 7.10 (t, *J* = 5.7 Hz, 1H, $CH_2CH{=}N$), 6.83–6.89 (m, 2H, phenyl H-3, H-5), 5.05 (br s, 1H, N*H*), 3.90 (d, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$), 3.80 (s, 3H, OCH_3), 3.51 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.27 (t, *J* = 2.4 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 158.40 (phenyl C-4), 143.10 (*C*H=N), 129.82 (phenyl C-2, C-6), 129.58 (phenyl C-1), 114.03 (phenyl C-3, C-5), 72.15 (*C*≡CH), 61.10 (C≡CH), 55.30 (OCH_3), 39.10 ($CH_2C{\equiv}CH$), 38.12 ($CH_2CH{=}N$); **(*Z*)-8l isomer:** ^{1}H NMR ($CDCl_3$): δ 7.15–7.19 (m, 2H, phenyl H-2, H-6), 6.83–6.89 (m, 2H, phenyl H-3, H-5), 6.79 (t, *J* = 5.1 Hz, 1H, $CH_2CH{=}N$), 5.05 (br s, 1H, N*H*), 3.99 (d, *J* = 2.1 Hz, 2H, $CH_2C{\equiv}CH$), 3.81 (s, 3H, OCH_3), 3.41 (d, *J* = 5.1 Hz, 2H, $CH_2CH{=}N$), 2.29 (t, *J* = 2.1 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 158.40 (phenyl C-4), 143.10 (*C*H=N), 129.55 (phenyl C-2, C-6), 129.58 (phenyl C-1), 114.30 (phenyl C-3, C-5), 72.15 (*C*≡CH), 61.10 (C≡CH), 55.30 (OCH_3), 40.45 ($CH_2C{\equiv}CH$), 23.38 ($CH_2CH{=}N$).

5.3.13. (*E*)-*N*-Bis-(2-propynyl)-2-(4-chlorophenyl)ethyidene]-hydrazine [(*E*)-7m], and (*E*)- and (*Z*)-*N*-(2-Propynyl)-2-(4-chlorophenyl)ethylidene]hydrazines [(*E*)-8m and (*Z*)-8m]

(*E*)-7m: Yellow oil separated using petroleum ether–ethyl acetate as eluant (97:3, v/v); yield, 16%; IR (liquid film): 3302 (C≡CH), 1727 (CH=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.28 (d, *J* = 8.4 Hz, 2H, phenyl H-3, H-5), 7.18 (d, *J* = 8.4 Hz, 2H, phenyl H-2, H-6), 7.00 (t, *J* = 5.7 Hz, 1H, $CH_2CH{=}N$), 3.97 (d, *J* = 2.4 Hz, 4H, $CH_2C{\equiv}CH$), 3.58 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.26 (t, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 140.97 (*C*H=N), 136.15 (phenyl C-1), 132.17 (phenyl C-4), 130.87 (phenyl C-2, C-6), 128.59 (phenyl C-3, C-5), 73.52 (*C*≡CH), 61.30 (C≡*C*H), 42.18 ($CH_2C{\equiv}CH$), 38.85 ($CH_2CH{=}N$).

(*E*)-8m and (*Z*)-8m (ratio 4.1:1): Yellow oil separated using petroleum ether–ethyl acetate (93:7, v/v) as eluant; yield, 32%; IR (liquid film): 3299 (C≡CH), 1698 (CH=N) cm^{-1}; **(*E*)-8m isomer:** ^{1}H NMR ($CDCl_3$): δ 7.28 (d, *J* = 8.4 Hz, 2H, phenyl H-3, H-5), 7.16 (d, *J* = 8.4 Hz, 2H, phenyl H-2, H-6), 7.07 (t, *J* = 5.4 Hz, 1H, $CH_2CH{=}N$), 5.10 (br s, 1H, N*H*), 3.89 (d, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$), 3.53 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.26 (t, *J* = 2.4 Hz,1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 142.5 (phenyl C-1), 141.60 (*C*H=N), 136.00 (phenyl C-4), 130.08 (phenyl C-2, C-6), 128.59 (phenyl C-3, C-5), 72.50 (*C*≡CH), 60.30 (C≡*C*H), 38.91 ($CH_2C{\equiv}CH$), 38.22 ($CH_2CH{=}N$); **(*Z*)-8m isomer:** ^{1}H NMR ($CDCl_3$): δ 7.28 (d, *J* = 8.4 Hz, 2H, phenyl H-3, H-5), 7.16 (d, *J* = 8.4 Hz, 2H, phenyl H-2, H-6), 6.75 (t, *J* = 4.8 Hz, 1H, $CH_2CH{=}N$), 5.10 (br s, 1H, N*H*), 3.98 (d, *J* = 2.1 Hz, 2H, $CH_2C{\equiv}CH$), 3.42 (d, *J* = 4.8 Hz, 2H, $CH_2CH{=}N$), 2.29 (t, *J* = 2.1 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 142.5 (phenyl C-1), 141.60 (*C*H=N), 136.00 (phenyl C-4), 128.90 (phenyl C-2, C-6), 128.80 (phenyl C-3, C-5), 72.50 (*C*≡CH), 60.30 (C≡*C*H), 38.91 ($CH_2C{\equiv}CH$), 38.22 ($CH_2CH{=}N$).

5.3.14. (*E*)- and (*Z*)-*N*-(2-Propynyl)-2-(4-fluorophenyl)ethylidene]hydrazines [(*E*)-8n and (*Z*)-8n, ratio 3.9:1]

Yellow oil separated using petroleum ether–ethyl acetate (92:8, v/v) as eluant; yield, 21%; IR (liquid film): 3290 (C≡CH), 1700 (CH=N) cm^{-1}; **(*E*)-8n isomer:** ^{1}H NMR ($CDCl_3$): δ *7.14–7.24* (m, 2H, phenyl H-2, H-6), 7.16 (t, *J* = 6.0 Hz, 1H, $CH_2CH{=}N$), 6.92–7.01 (m, 2H, phenyl H-3, H-5), 5.03 (br s, 1H, N*H*), 3.54 (d, *J* = 6.0 Hz, 2H, $CH_2CH{=}N$), 2.27 (t, *J* = 2.4 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 163.26 (d, $^{1}J_{C,F}$ = 243.89 Hz, C_4 aryl), 142.18 (*C*H=N), 135.00 (phenyl C-1), 130.22 (d, $^{3}J_{C,F}$ = 7.69 Hz, phenyl C-2, C-6), 115.35 (d, $^{2}J_{C,F}$ = 20.88 Hz, phenyl C-3, C-5), 72.19 (*C*≡CH), 60.00 (C≡*C*H), 39.02 ($CH_2C{\equiv}CH$), 38.17 ($CH_2CH{=}N$); **(*Z*)-8n isomer:** ^{1}H NMR ($CDCl_3$): δ 7.14–7.24 (m, 2H, phenyl H-2, H-6), 6.92–7.01 (m, 2H, phenyl H-3, H-5), 6.77 (t, *J* = 4.8 Hz, 1H, $CH_2CH{=}N$), 5.03 (br s, 1H, N*H*), 3.99 (d, *J* = 2.1 Hz, 2H, $CH_2C{\equiv}CH$), 3.43 (d, *J* = 4.8 Hz, 2H, $CH_2CH{=}N$), 2.30 (t, *J* = 2.1 Hz, 1H, $CH_2C{\equiv}CH$); ^{13}C NMR ($CDCl_3$): δ 163.26 (d, $^{1}J_{C,F}$ = 243.89 Hz, phenyl C-4), 142.18 (*C*H=N), 135.00 (phenyl C-1), 130.20 (d, $^{3}J_{C,F}$ = 7.69 Hz, phenyl C-2, C-6), 115.23 (d, $^{2}J_{C,F}$ = 20.88 Hz, phenyl C-3, C-5), 72.19 (*C*≡CH), 60.00 (C≡CH), 40.50 ($CH_2C{\equiv}CH$), 31.50 ($CH_2CH{=}N$).

5.3.15. (*E*)- and (*Z*)-*N*-(2-Propynyl)-2-(4-trifluoromethylphenyl)ethylidene]hydrazines [(*E*)-8o and (*Z*)-8o, ratio 5.4:1]

Yellow oil separated using petroleum ether–ethyl acetate (90:10, v/v) as eluant; yield, 22%; IR (liquid film): 3295 (C≡CH), 1645 (CH=N) cm^{-1}; **(*E*)-8o isomer:** ^{1}H NMR ($CDCl_3$): δ 7.54–7.62 (m, 2H, phenyl H-3, H-5), 7.30–7.38 (m, 2H, phenyl H-2, H-6), 7.10 (t, *J* = 5.7 Hz, 1H, $CH_2CH{=}N$), 5.06 (br s, 1H, N*H*), 3.91 (d, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$), 3.63 (d, *J* = 5.7 Hz, 2H, $CH_2CH{=}N$), 2.28 (t, *J* = 2.4 Hz, 1H, $CH_2C{\equiv}CH$); **(*Z*)-8o isomer:** ^{1}H NMR ($CDCl_3$): δ *7.54–7.62* (m, 2H, phenyl H-3, H-5), 7.30–7.38 (m, 2H, phenyl H-

2, H-6), 6.77 (t, J = 4.8 Hz, 1H, CH_2CH=N), 5.06 (br s, 1H, NH), 4.00 (d, J = 2.1 Hz, 2H, CH_2C≡CH), 3.52 (d, J = 4.8 Hz, 2H, CH_2CH=N), 2.31 (t, J = 2.1 Hz, 1H, CH_2C≡CH.

5.3.16. (*E*)-*N*-Bis-(2-propynyl)-2-(phenyl)ethyidene]hydrazine [(*E*)-7p], and (*E*)- and (*Z*)-*N*-(2-propynyl)-2-(phenyl)ethylidene]-hydrazines [(*E*)-8p and (*Z*)-8p]

(*E*)-7p: Yellow oil separated using petroleum ether–ethyl acetate as eluant (96:4, v/v); yield, 16%; IR (liquid film): 3299 (C≡CH), 1726 (CH=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.21–7.33 (m, 5H, phenyl hydrogens), 7.05 (t, J = 5.4 Hz, 1H, CH_2CH=N), 3.98 (d, J = 2.4 Hz, 4 H, CH_2C≡CH), 3.62 (d, J = 5.4 Hz, 2 H, CH_2CH=N), 2.27 (t, J = 2.4 Hz, 2H, CH_2C≡CH); ^{13}C NMR ($CDCl_3$): δ 158.89 (*C*H=N), 137.21 (phenyl C-1), 128.39 (phenyl C-2, C-6), 128.04 (phenyl C-3, C5), 126.00 (phenyl C-4) 73.01 (*C*≡CH), 61.00 (C≡*C*H), 41.76 (*C*H_2C≡CH), 39.07 (*C*H_2CH=N).

(*E*)-8p and (*Z*)-8p (ratio 3.4:1): Yellow oil separated using petroleum ether–ethyl acetate (92:8, v/v) as eluant; yield, 32%; IR (liquid film): 3281 (C≡CH), 1649 (CH=N) cm^{-1}; **(*E*)-8p isomer:** ^{1}H NMR ($CDCl_3$): δ 7.22–7.37 (m, 5H, phenyl hydrogens), 7.13 (t, J = 5.7 Hz, 1H, CH_2CH=N), 5.08 (br s, 1H, NH), 3.90 (d, J = 2.7 Hz, 2H, CH_2C≡CH), 3.57 (d, J = 5.7 Hz, 2H, CH_2CH=N), 2.27 (t, J = 2.7 Hz, 1H, CH_2C≡CH); ^{13}C NMR ($CDCl_3$): δ 142.63 (*C*H=N), 137.37 (phenyl C-1), 128.84 (phenyl C-2, C-6), 128.58 (phenyl C-3, C-5), 126.54 (phenyl C-4), 72.16 (*C*≡CH), 60.15 (C≡*C*H), 39.07 (*C*H_2C≡CH), 39.00 (*C*H_2CH=N); **(*Z*)-8p isomer:** ^{1}H NMR ($CDCl_3$): δ 7.22–7.37 (m, 5H, phenyl hydrogens), 6.82 (t, J = 4.8 Hz, 1H, CH_2CH=N), 5.08 (br s, 1H, NH), 3.99 (d, J = 2.4 Hz, 2H, CH_2C≡CH), 3.47 (d, J = 4.8 Hz, 2H, CH_2CH=N), 2.30 (t, J = 2.4 Hz, 1H, CH_2C≡CH); ^{13}C NMR ($CDCl_3$): δ 143.17 (*C*H=N), 137.37 (phenyl C-1), 128.84 (phenyl C-2, C-6), 128.58 (phenyl C-3, C-5), 126.79 (phenyl C-4), 71.83 (*C*≡CH), 60.15 (C≡*C*H), 40.45 (*C*H_2C≡CH), 33.29 (*C*H_2CH=N).

5.4. Neurochemical studies

5.4.1. Animals

Male Sprague–Dawley rats (approximately 300 g) were pair-housed in polycarbonate cages with free access to food (Purina Rat Chow) and water, and were maintained on a 12-h light–dark cycle (lights on at 0800). All animal procedures were approved by the University of Alberta Biosciences Animal Policy and Welfare Committee, and were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

In the ex vivo studies, animals were injected intraperitoneally with equimolar amounts of PEH (30 mg/kg), **7p** (47 mg/kg), or **8p** (38.5 mg/kg) or vehicle (corn oil) and were killed by decapitation 3, 6, or 12 h following drug administration. Brains were rapidly removed and flash-frozen in 2-methylbutane on solid carbon dioxide, and were stored at −80 °C. Subsequently, partially thawed brains were homogenized in 5 volumes of ice-cold distilled water, and stored in aliquots at −80 °C for use in subsequent analyses (GABA-T, MAO-A, MAO-B, and brain levels of amino acids) after addition to the appropriate buffer medium.

5.4.2. GABA-T

A modification of the colorimetric method of Sethi[49] was used in the initial in vitro screen. GABASE was incubated with the drug of interest and an incubation buffer. In the ex vivo study of homogenates from brains of rats treated with the analogs, GABA-T activity was measured using a modification[18] of the radiochemical procedure of Sterri and Fonnum.[50] Briefly, homogenates were incubated with radiolabeled GABA in a buffer containing pyridoxal phosphate and the resultant products were isolated using a liquid anion exchanger (tri-*n*-octylamine). Radioactivity (dpm) was counted using a Beckman LS 7500 liquid scintillation spectrometer.

5.4.3. MAO

Monoamine oxidase activity was determined using a modified protocol described by Lyles and Callingham.[51] In the in vitro studies, the drugs were pre-incubated with control rat brain homogenate prior to conducting the assay. Briefly, tissue homogenate was diluted in potassium phosphate buffer (0.2 M, pH 7.4), and an aliquot (50 μl) was incubated with the appropriate radiolabeled substrates (^{14}C-labeled 5-hydroxytryptamine and β-phenylethylamine were used as substrates for MAO-A and –B, respectively). The reaction products were extracted by a mixture of ethyl acetate and toluene (1:1 v/v), and a portion of the top layer was added to a vial containing scintillation fluid for radioactivity measurement. Radioactivity (dpm) was counted using a Beckman LS 7500 liquid scintillation spectrometer.

5.4.4. Amino acid analysis using HPLC

Amino acid levels (GABA, glutamate, alanine and glycine) were determined using a modified procedure previously described by Grant and colleagues[52] for the quantification of amino acids using HPLC combined with fluorescence detection, following derivatization with fluoraldehyde reagent [o-phthaldialdehyde (OPA)] and isobutyryl-L-cysteine (IBLC). Briefly, a portion of the homogenate (100 μl) was added to ice-cold methanol (400 μl), re-homogenized and centrifuged (13,000*g*, 4 °C). The supernatants were further diluted by a factor of 2 in ice-cold water, and transferred to HPLC vials. A portion of the supernatant (5 μl) was reacted with OPA/IBLC (5 μl) in the injection loop of a Waters Alliance 2690XE system for 1.5 min before injection onto the analytical column (Symmetry C_{18} 5μm (4.6 × 150 mm)), connected to a Symmetry C_{18} guard column, held at 30 °C. A Waters 474 fluorescence detector was set to an excitation wavelength of 344 nm and an emission wavelength of 443 nm. Data were collected and analyzed using the Empower Pro software package (Waters).

5.4.5. Statistics

Data were analyzed by analysis of variance (ANOVA), followed by the Newman–Keuls test. Statistical significance was established using a probability value of <0.05.

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