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Novel Arylalkenylpropargylamines as Neuroprotective, Potent, and Selective Monoamine Oxidase B Inhibitors for the Treatment of Parkinson's Disease

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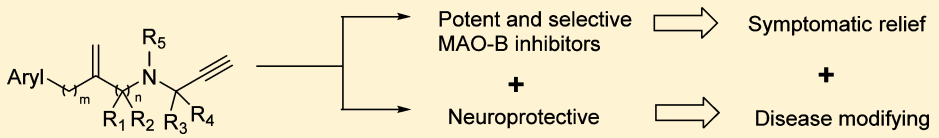
Supporting Information



ABSTRACT: To develop novel neuroprotective agents, a library of novel arylalkenylpropargylamines was synthesized and tested for inhibitory activities against monoamine oxidases. From this, a number of highly potent and selective monoamine oxidase B inhibitors were identified. Selected compounds were also tested for neuroprotection in in vitro studies with PC-12 cells treated with 6-OHDA and rotenone, respectively. It was observed that some of the compounds tested yielded a marked increase in survival in PC-12 cells treated with the neurotoxins. This indicates that these propargylamines are able to confer protection against the effects of the toxins and may also be considered as novel disease-modifying anti-Parkinsonian agents, which are much needed for the therapy of Parkinson's disease.

INTRODUCTION

Parkinson's disease (PD) is a debilitating, progressive, neurodegenerative disorder that predominantly affects the elderly population. It is the second most common neurodegenerative disease after Alzheimer's disease and is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. In fact, most therapies are aimed at increasing the concentration of dopamine in the brain. L-DOPA (Figure 1), itself or in combination with a peripheral L-DOPA decarboxylase inhibitor, is utilized predominantly for dopamine replacement, being a prodrug that increases the concentration of dopamine. Other strategies include the use of inhibitors that reduce the metabolism of dopamine, such as catechol-*O*-methyl transferase (COMT) and monoamine oxidase (MAO) inhibitors. Despite the many advances in the treatment of PD to date, these therapies are only symptomatic in nature. However, in addition to symptomatic relief, what is urgently lacking is a therapy that is able to halt disease progression. As the etiology and pathomechanism of PD are complex and not fully understood, such a therapy would necessarily require protection of the dopaminergic neurons from cell death. To illustrate some advances in this regard, selegiline, a selective MAO-B inhibitor that is used in early stage of PD, has been demonstrated to have antioxidant and protective effects in certain experimental models of PD.[1,2] However, selegiline is metabolized to L-amphetamine and L-methamphetamine, which though less psychoactive than their D-enantiomers, can result in cardiovascular implications and, at higher concentrations (>10^{-7} M), can increase the rate of apoptosis.[3]

A more recently FDA-approved drug for PD, rasagiline, is an even more selective MAO-B inhibitor[4] as compared to selegiline, and as its lacks amphetamine-like metabolites, it may offer a therapeutic advantage over selegiline. In addition, several studies have shown that rasagiline, due also to its propargylamine moiety, is able to counteract various steps of

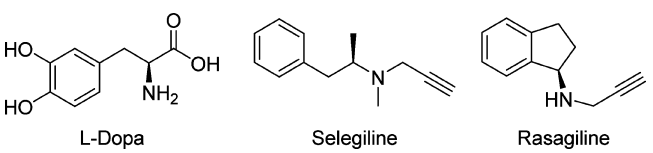


Figure 1. Drugs for the treatment of Parkinson's disease.

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the apoptotic cascade, which ultimately prevents cell death. These include activation of caspase 3 and poly(ADP-ribose) polymerase-1 (PARP-1), translocation of glyceraldehyde-3-phosphate dehydrogenase (GADPH), and nucleosomal DNA fragmentation.[5] Rasagiline is also able to induce the expression of antiapoptotic proteins Bcl-2 and Bcl-X_L, while pro-apoptotic Bad and Bax proteins are downregulated.[6−10] Despite these promising findings and other studies on the neuroprotective properties of rasagiline, clinical trials have failed to show a clear neuroprotective action, and this has consequently led to the rejection of rasagiline by the FDA[11] as a disease-modifying drug for PD. The fate of rasagiline further exemplifies the urgent need for the development of an anti-Parkinsonian drug that is not only selective in MAO-B inhibition but also possesses clear, clinically proven neuroprotective action to address the unmet medical need in PD.

To this end, our strategy was to design multifunctional and multitargeted novel propargylamines with potent and selective MAO-B activity and neuroprotective effects. The design of our novel propargylamines is based on the knowledge that the propargylamino group is likely to be important in neuroprotection, while MAO-B selectivity and potency as well as overall neuroprotective profile can be tuned through judicious choice of the skeleton and its substituents. In addition, recent reports suggest that MAO-B inhibitors can have neuroprotective functions.[12] Herein we describe the synthesis of a library of novel compounds with the general structure shown in Figure 2. The test compounds were screened for MAO-A and MAO-B inhibition, in both rat and human enzyme assays, as well as in neurotoxin-treated PC12 cells, a widely accepted model for neuroprotection.

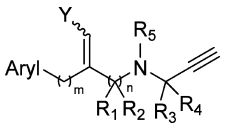
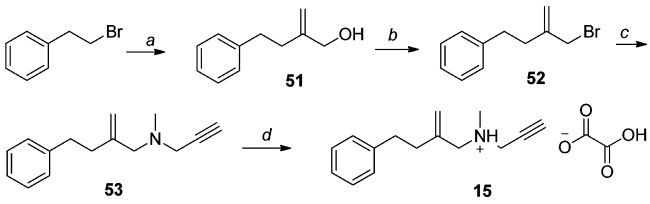


Figure 2. General structure of target compounds.

RESULTS AND DISCUSSION

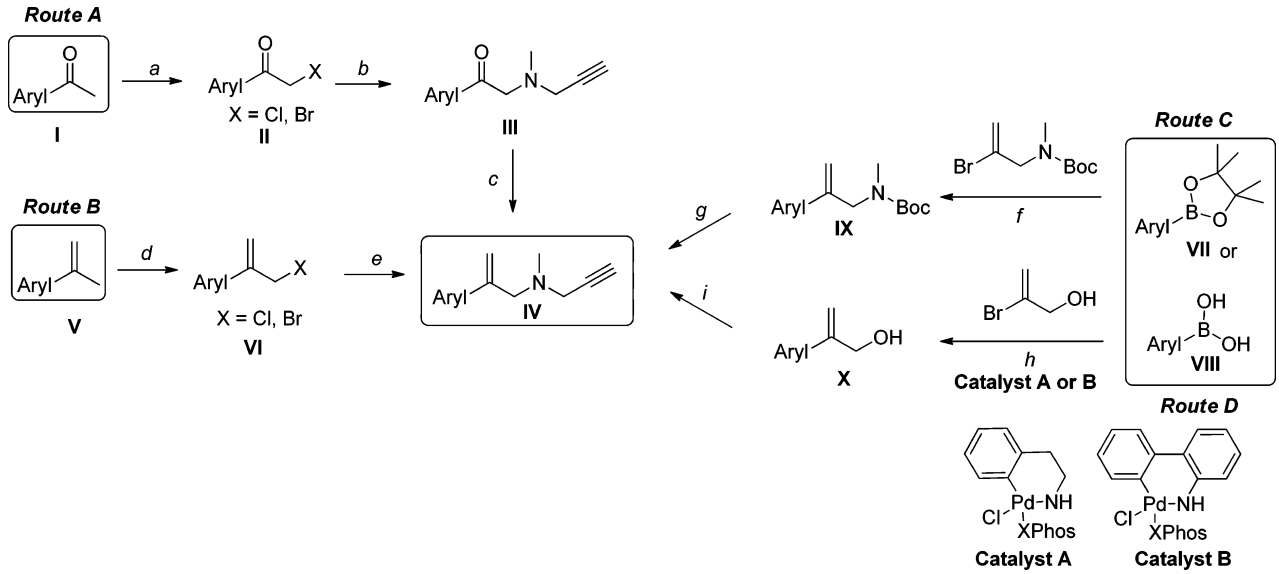
Synthetic Chemistry. The compounds synthesized and studied in the biological assays, comprising an (hetero)aryl moiety, a vinyl, and backbone substituents, can be conveniently divided into four groups. Groups 1 and 2 are comprised of compounds with a variety of unsubstituted aryl and heteroaryl groups, while in group 3, compounds bear mono substitution on the aryl ring and those in group 4 have disubstituted aryl rings. The target compounds **1**−**50** (except **15** and **18**) were synthesized using the four general synthetic routes shown in Scheme 1. Compounds **15** and **18** were obtained using the synthetic routes shown in Schemes 2 and 3, respectively. The choice of synthetic routes was based on the availability of starting material/chemical reagents and the potential for formation of side products. The first route (route A) started from the commercially available aryl methyl ketone **I**, which

Scheme 2. Synthesis of Compound 15[a]



[a]Reagents and conditions: (a) Mg, I_2, ether, 1 h then propargyl alcohol, CuI, 40 °C, 16 h, 67%; (b) CBr_4, PPh_3, DCM, 16 h, 86%; (c) *N*-methylpropargylamine, DCM, 16 h, 55%; (d) oxalic acid, ^{i}PrOH, 83%.

Scheme 1. General Synthetic Routes to Target Compounds[a]



[a]Reagents and conditions: (a) Br_2, AcOH, toluene, 0 °C to rt or benzyltrimethylammonium dichloroiodate, THF; (b) *N*-methylpropargylamine, ether, acetonitrile (ACN) or (dichloromethane) DCM; (c) $TMSCHN_2$, ^{i}PrOH, $(Ph_3P)_3RhCl$, PPh_3, dioxane, 60 °C; (d) diphenyl diselenide, *N*-chlorosuccinimide (NCS), DCM, or NBS, chlorobenzene, 160 °C in an oil bath or 180 °C in a microwave reactor; (e) *N*-methylpropargylamine, ^{i}PrOH; (f) $Pd(OAc)_2$, XPhos, $K_3PO_4·H_2O$, $EtOH/H_2O$, 60 °C; (g) TFA/DCM then propargyl bromide, Na_2CO_3, DCM; (h) Buchwald's precatalyst (catalyst A or B), $K_3PO_4·H_2O$, THF/H_2O, 60 °C; (i) MsCl, NEt_3, DCM, then *N*-methylpropargylamine.

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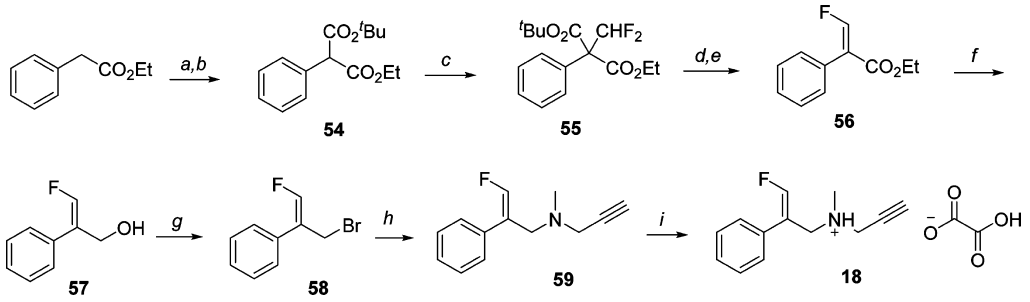
Scheme 3. Synthesis of Compound 18[a]



[a]Reagents and conditions: (a) LDA, CO_2, THF; (b) DCC, DMAP, ^{t}BuOH, DCM, 16 h, 81% over two steps; (c) NaO^{t}Bu, Freon 22 ($ClCHF_2$), 9 bar, THF, 16 h, 86%; (d) TFA, DCM, 40 min; (e) NaOH, 15 min, 68% over two steps; (f) DIBALH, THF, 1 h, 68%; (g) CBr_4, PPh_3, DCM, 16 h, 60%; (h) *N*-methylpropargylamine, ACN, 16 h, 60%; (i) oxalic acid, ^{i}PrOH, 67%.

was converted to the aryl halo ketone **II**.[13] Subsequently, the nucleophilic substitution was achieved using *N*-methylpropargylamine, followed by a modified Wittig olefination[14,15] reaction to provide the desired tertiary amine **IV**. However, low yields were frequently obtained in the olefination protocol and the toxicity of $TMSCHN_2$ prohibited large-scale synthesis. As outlined in route B, an alternative procedure utilized the commercially available aryl methylstyrene **V**. Allylic bromination or chlorination followed by nucleophilic substitution of the halide with *N*-methylpropargylamine gave the desired compound **IV**. However, in some instances, allylic bromination using NBS alone or NCS and diphenyl diselenide[16] led to a mixture of allylic and vinyl halides. The use of catalytic amount of $Yb(OTf)_3$ and TMSCl in this step alleviated these regioselectivity issues in some cases.[17]

In order to facilitate compound library synthesis, a modular approach was sought. Suzuki cross-coupling reactions (routes C and D) could be utilized depending on the availability of the precursors. In route C, the Suzuki coupling between the aryl boronate ester **VII** or aryl boronic acid **VIII** with *tert*-butyl (2-bromoallyl)methylcarbamate proceeded in the presence of $Pd(OAc)_2$, XPhos, and K_3PO_4 as the base in a 2:1 $EtOH/H_2O$ mixture.[18] The resultant allylic amine **IX** was subjected to Boc deprotection followed by alkylation using propargyl bromide. In route D, Suzuki coupling was carried out between the aryl boronate ester **VII** or aryl boronic acid **VIII** with 2-bromoallyl alcohol in the presence of Buchwald's precatalyst (catalyst A or B) and K_3PO_4 as the base.[19,20] The resultant alcohol **X** was subjected to mesylation and amination in a one-pot reaction to give the desired arylalkenyl *N*-methylpropargylamines. In cases where compounds bear an electron-donating group at the para position of the aryl group, the target compounds can only be accessed via route D. This is due to the formation of oxazolidinones during the Boc deprotection step of route C. All the propargyl amines **IV** (except compound **13**, which was used as the free base) were converted to the final form, either the HCl salt or oxalate salt.

Scheme 2 depicts the synthetic route that was utilized for compound **15**. Using copper catalysis, phenylethylmagnesium bromide was added in a regioselective fashion to propargyl alcohol.[21] The resultant allylic alcohol **51** underwent the Appel reaction[22] followed by nucleophilic substitution to furnish the desired amine **53**. The free base was then converted to the oxalate salt **15**.

The synthetic route of the vinyl fluoride analog **18** is outlined in Scheme 3. The mixed malonate **54** was prepared in two steps from commercially available ethyl 2-phenylacetate. The introduction of a difluoromethyl group in **55** was achieved when a THF solution of **54** was stirred in the presence of NaO^{t}Bu under an atmosphere of Freon 22 ($ClCHF_2$) at 9 bar for 16 h.[23] Decarboxylative fluoride elimination occurred in two steps from **55** to afford the vinyl fluoride **56**. The acrylate ester **56** was then reduced using DIBAL to provide the allylic alcohol **57**. Appel reaction[22] of the resultant allylic alcohol **57**, followed by nucleophilic substitution, furnished the desired product **59**, which was converted to the oxalate salt **18**.

A summary of the synthesized compounds in groups 1−4 along with their characteristics and method of synthesis is shown in Table 1.

Biological Assays. Initial preliminary screening for MAO-A and MAO-B enzyme inhibitory activities was carried out for the majority of the compounds using rat MAO enzymes. Potent and selective inhibitors were subsequently screened against isolated human MAO-A and MAO-B enzymes. Selected compounds from groups 1−4 were also screened for neuroprotective properties in PC12 cells.

Rat MAO-A and MAO-B Inhibitory Activities. Rat MAO activities were determined on the rat brain homogenates with ^{14}C-5-serotonin (MAO-A) or ^{14}C-phenylethylamine (MAO-B) substrates (Table 2). Rasagiline displayed rMAO-B inhibition with an IC_{50} of 1 nM (selectivity 3000). From group 1 compounds, compounds **1**, **6**, and **7**, bearing phenyl, 3-furanyl, and 2-thiophenyl moieties, were potent and selective MAO-B inhibitors. However, substitution using pyridyl (**2**−**4**), 3-thiophenyl (**8**), and thiazolyl (**9**) moieties resulted in weak MAO-B inhibitors.

Next, in group 2 compounds, it was observed that both the *N*-desmethyl (**10**) and *N*-ethyl (**11**) analogues are micromolar inhibitors of rMAO-B. The introduction of a methyl group in either position of the methylene group (**12** and **13**) was generally detrimental to rMAO-B inhibition activity. Compound **14**, with an increase in the distance (n = 3) between the alkene and the propargyl moiety of the structure as compared to compound **1**, was inactive. Interestingly, increasing the distance between the aryl ring and the alkene moiety (m = 2 for **15**, m = 1 for **16**) gave comparable rMAO-B inhibitory activity as compared to the parent compound **1**. Replacing the propargyl moiety with an allylic moiety (in **17**) resulted in significant loss of activity. Masking the alkene moiety by a bioisosteric fluorine replacement (in **18**) gave a micromolar selective rMAO-B inhibitor.

In group 3, the effect of monosubstitution on the benzene ring was explored. Substitutions with a *p*-halogen resulted in the more active rMAO-B inhibitors (4-chloro, 4-bromo, and 4-

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Table 1. Final Salt Form of the Test Compounds

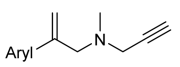
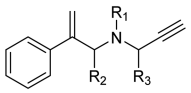
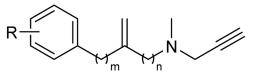
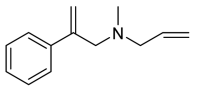
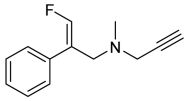
Compound		Route	Salt	Molecular formula	Melting point (°C)
	GROUP 1 				
1	Aryl = phenyl	B	HCl	$C_{12}H_{16}ClN$	166 – 167
2	Aryl = 2-pyridyl	A	Oxalate	$C_{14}H_{16}N_2O_4$	113 – 114
3	Aryl = 3-pyridyl	A	Oxalate	$C_{16}H_{18}N_2O_8$	149 – 150
4	Aryl = 4-pyridyl	A	Oxalate	$C_{16}H_{18}N_2O_8$	158 – 160
5	Aryl = 2-furanyl	A	Oxalate	$C_{13}H_{15}NO_5$	121 – 122
6	Aryl = 3-furanyl	A	Oxalate	$C_{13}H_{15}NO_5$	116 – 118
7	Aryl = 2-thiophenyl	B	HCl	$C_{11}H_{14}ClNS$	155
8	Aryl = 3-thiophenyl	A	Oxalate	$C_{13}H_{15}NO_4S$	125 – 126
9	Aryl = 2-thiazolyl	A	Oxalate	$C_{12}H_{14}N_2O_4S$	153 – 154
	GROUP 2 				
10	$R_1 = R_2 = R_3 = H$	B	HCl	$C_{13}H_{14}ClN$	135
11	R_1 = Et, $R_2 = R_3 = H$	C	Oxalate	$C_{16}H_{19}NO_4$	85 – 87
12	$R_1 = R_3$ = Me, $R_2 = H$	D	Oxalate	$C_{16}H_{19}NO_4$	109 – 110
13	$R_1 = R_3 = H$, R_2 = Me	A	Free base	$C_{14}H_{17}N$	n.a.
					
14	m = 0, n = 3, R = H	A	Oxalate	$C_{17}H_{21}NO_4$	96 – 97
15	m = 2, n = 1, R = H	Scheme 2	Oxalate	$C_{17}H_{21}NO_4$	117 – 118
16	m = 1, n = 1, R = 3-F, 4-F	A	Oxalate	$C_{16}H_{17}F_2NO_4$	97 – 99
17		B	HCl	$C_{13}H_{18}ClN$	127 – 128
18		Scheme 3	Oxalate	$C_{15}H_{16}FNO_4$	153 – 155

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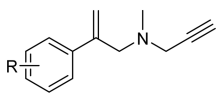
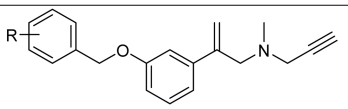
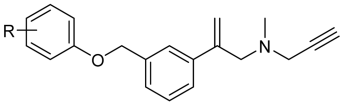
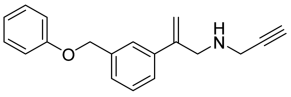
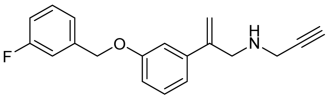
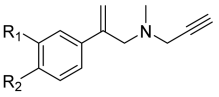
Table 1. continued

Compound		Route	Salt	Molecular formula	Melting point (°C)
	GROUP 3 				
19	R = 3-Cl	A	Oxalate	$C_{15}H_{16}ClNO_4$	142 – 144
20	R = 4-Cl	B	Oxalate	$C_{15}H_{16}ClNO_4$	160 – 161
21	R = 3-Br	A	Oxalate	$C_{15}H_{16}BrNO_4$	130 - 132
22	R = 4-Br	B	HCl	$C_{13}H_{15}BrClN$	176 – 177
23	R = 2-F	B	HCl	$C_{13}H_{15}FClN$	140
24	R = 3-F	C	Oxalate	$C_{15}H_{16}FNO_4$	142 – 143
25	R = 4-F	B	HCl	$C_{13}H_{15}FClN$	182 – 183
26	R = 2-OMe	B	HCl	$C_{14}H_{18}ClNO$	135
27	R = 3-OMe	D	Oxalate	$C_{16}H_{19}NO_5$	106 – 107
28	R = 4-OMe	B	HCl	$C_{14}H_{18}ClNO$	148 – 149
29	R = 3-OCH_2Ph	C	Oxalate	$C_{22}H_{23}NO_5$	112 – 113
30	R = 4-OCH_2Ph	D	Oxalate	$C_{22}H_{23}NO_5$	138 – 139
31	R = 3-CH_2OPh	C	Oxalate	$C_{22}H_{23}NO_5$	126 – 127
32	R = 4-CH_2OPh	C	Oxalate	$C_{22}H_{23}NO_5$	147 – 150
33	R = 3-CH_2NHPh	D	Oxalate	$C_{22}H_{24}N_2O_4$	143 – 147
34	R = 4-CH_2NHPh	D	Oxalate	$C_{22}H_{24}N_2O_4$	140 – 141

DOI: 10.1021/jm501722s
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. continued

Compound		Route	Salt	Molecular formula	Melting point (°C)
					
35	R = 3-F	D	Oxalate	$C_{22}H_{22}FNO_5$	100 – 102
36	R = 4-F	D	Oxalate	$C_{22}H_{22}FNO_5$	118 – 119
37	R = 3-OMe	D	Oxalate	$C_{23}H_{25}NO_6$	113 – 114
38	R = 4-OMe	D	Oxalate	$C_{23}H_{25}NO_6$	104 – 105
					
39	R = 3-F	D	Oxalate	$C_{22}H_{22}FNO_5$	126 – 127
40	R = 4-F	D	Oxalate	$C_{22}H_{22}FNO_5$	111 – 112
41	R = 3-OMe	D	Oxalate	$C_{23}H_{25}NO_6$	115 – 116
42	R = 4-OMe	C	Oxalate	$C_{23}H_{25}NO_6$	118 – 119
43		D	Oxalate	$C_{21}H_{21}NO_5$	185 – 187
44		D	Oxalate	$C_{21}H_{20}FNO_5$	182 – 184
	GROUP 4 				
45	$R_1 = R_2 = Me$	C	Oxalate	$C_{17}H_{21}NO_4$	136 – 137
46	$R_1 = R_2 = F$	A	Oxalate	$C_{15}H_{15}F_2NO_4$	144 – 145
47	$R_1 = R_2 = Cl$	D	Oxalate	$C_{15}H_{15}Cl_2NO_4$	153 – 156
48	$R_1 = Me, R_2 = F$	C	Oxalate	$C_{16}H_{18}FNO_4$	156 – 158
49	$R_1 = Me, R_2 = Cl$	D	Oxalate	$C_{16}H_{18}ClNO_4$	157 – 158
50	$R_1 = Cl, R_2 = F$	D	Oxalate	$C_{15}H_{15}ClFNO_4$	145 – 146

fluorobenzene compounds **20**, **22**, and **25**), when compared to other halogen-substituted regioisomers. Next, we investigated the effect of the methoxy and benzyloxy substitution on the aromatic ring, synthesizing all possible regioisomers. The 2-methoxyphenyl propargylamine **26** was found to be a less selective rMAO-B inhibitor. However, substitution of the methoxy and benzyloxy group at the meta- and para-positions (compounds **27**–**30**) resulted in submicromolar rMAO-B inhibitors. Interestingly, the meta- and para-substituted phenoxymethylene compounds **31** and **32** are selective nanomolar inhibitors of rMAO-B, especially compound **31** (IC_{50} = 1.5 nM). Substitution at ring B of compounds **29** and **31** afforded compounds **35**–**42**; further studies revealed that compound **35** (with a 3-fluoro substitution in ring B) is a potent and selective rMAO-B inhibitor (IC_{50} = 2.0 nM and selectivity 2500, which are comparable to rasagiline).

For group 4 compounds, we investigated the effects of disubstitution of the aromatic ring, especially at the meta- and para-position for the methyl, fluoro, and chloro substituents. Interestingly, all the compounds (**45**–**50**) were found to be

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Table 2. MAO-A and -B Inhibition Values of All Test Compounds Determined in the Rat Brain Assay

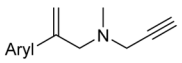
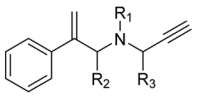
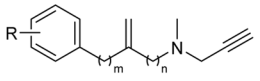
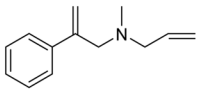
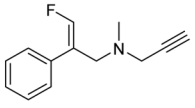
	Compound	rMAO-B, IC_{50}, nM	rMAO-A, IC_{50}, nM	Rat Selectivity, r(A/B)
	Rasagiline	1	3000	3000
	GROUP 1 			
1	Aryl = phenyl	50	2000	40
2	Aryl = 2-pyridyl	>100	N.D.[a]	
3	Aryl = 3-pyridyl	<1000	N.D.	
4	Aryl = 4-pyridyl	>1000	N.D.	
5	Aryl = 2-furanyl	100	3300	33
6	Aryl = 3-furanyl	33	800	24
7	Aryl = 2-thiophenyl	60	2500	41
8	Aryl = 3-thiophenyl	>100	N.D.	
9	Aryl = 2-thiazolyl	>100	N.D.	
	GROUP 2 			
10	$R_1 = R_2 = R_3 = H$	1000	10000	10
11	R_1 = Et, $R_2 = R_3 = H$	>1000	~100000	<100
12	$R_1 = R_2 = H$, R_3 = Me	~100000	>1000	>0.01
13	$R_1 = R_3 = H$, R_2 = Me	~1000	N.D.	
				
14	m = 0, n = 3, R = H	>1000	700	<0.7
15	m = 2, n = 1, R = H	60	660	11
16	m = 1, n = 1, R = 3-F, 4-F	100	~1000	~10
17		~100000	N.D.	
18		800	66000	82

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Table 2. continued

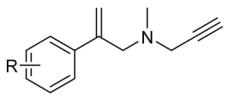
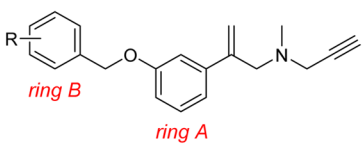
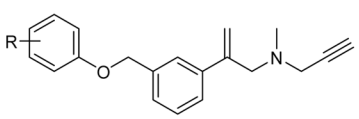
Compound		rMAO-B, IC_{50}, nM	rMAO-A, IC_{50}, nM	Rat Selectivity, r(A/B)
	GROUP 3 			
19	R = 3-Cl	90	1000	11
20	R = 4-Cl	25	2000	80
21	R = 3-Br	500	>1000	>2
22	R = 4-Br	20	2500	125
23	R = 2-F	500	5000	10
24	R = 3-F	60	2000	33
25	R = 4-F	33	660	20
26	R = 2-OMe	5000	5000	1
27	R = 3-OMe	90	1500	16.6
28	R = 4-OMe	80	200	2.5
29	R = 3-OCH_2Ph	20	5000	250
30	R = 4-OCH_2Ph	400	700	1.7
31	R = 3-CH_2OPh	1.5	400	266
32	R = 4-CH_2OPh	9	1500	16
33	R = 3-CH_2NHPh	60	>1000	>16.6
34	R = 4-CH_2NHPh	100	5000	50
				
35	R = 3-F	2	5000	2500
37	R = 3-OMe	9	>1000	>111
				
42	R = 4-OMe	6	700	116

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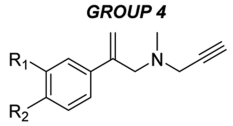
Table 2. continued

Compound		rMAO-B, IC_{50}, nM	rMAO-A, IC_{50}, nM	Rat Selectivity, r(A/B)
	GROUP 4 			
45	$R_1 = R_2 = Me$	80	1000	12.5
46	$R_1 = R_2 = F$	50	5000	100
47	$R_1 = R_2 = Cl$	80	~1000	~12.5
48	$R_1 = Me, R_2 = F$	70	4000	57
49	$R_1 = Me, R_2 = Cl$	80	~1000	~12.5
50	$R_1 = Cl, R_2 = F$	70	700	10

[a]N.D. = not determined.

selective, submicromolar inhibitors of rMAO-B. In particular, the 3,4-difluoroaryl compound **46** displayed the best selectivity and most potent rMAO-B inhibition activity of this group of compounds.

Human MAO-A and MAO-B Inhibitory Activities of Selected Compounds. The human MAO-A and MAO-B inhibitory activities were measured for selected compounds chosen from the preliminary screening above (Table 3). From group 1 compounds, it was observed that the 3-furanyl analog **6** was the most selective, subnanomolar hMAO-B inhibitor (IC_{50} = 0.72 nM with >2500-fold selectivity) followed by the 2-thiophenyl compound **7** (IC_{50} = 3.4 nM).

Meanwhile, from group 2, the secondary amine **10** resulted in micromolar hMAO-B inhibition, while the amines **15** and **16** (*m* = 2 for **15** and *m* = 1 for **16**) were submicromolar and poorly selective hMAO-B inhibitors, indicating the importance of methyl substitution at the amino group as well as the direct attachment of aryl group to the vinyl substituent.

From group 3 compounds, it was observed that para-substitution appeared to be optimal for activity, consistent with observations from the rat data, as can be observed in the 4-chloro, 4-bromo, and 4-fluorobenzene compounds (**20**, **22**, and **25**). Both 3-chloro- and 3-fluorobenzene compounds (**19** and **24**) were also potent hMAO-B inhibitors, although compound **19** was not a selective hMAO-B inhibitor. *m*-Benzyloxybenzene derivative **29** showed significant hMAO-B inhibition (IC_{50} = 6 nM, selectivity 350). Meanwhile, both meta- and para-substituted phenoxymethylenebenzene derivatives (**31** and **32**) were submicromolar hMAO-B inhibitors. We further explored derivatives of 3-benzyloxybenzene and the 3-phenoxymethylenebenzene compounds (**35**–**42**) and found that all meta- and para-substitution of fluoro and methoxy substituents in ring B resulted in moderate to excellent hMAO-B inhibitors. It was also interesting to note that the metabolite **44**, which is the *N*-desmethyl analogue of **35**, is a more potent and selective hMAO-B inhibitor (IC_{50} = 1.9 nM, selectivity >2200) as compared to the parent compound **35** (IC_{50} = 50 nM, selectivity >20).

The effects of disubstitution with fluoro, chloro, and methyl groups on the aromatic ring resulted in moderate to excellent hMAO-B inhibitors. The 3,4-difluorobenzene and 3,4-dichlorobenzene compounds (**46** and **47**) were identified as the most active hMAO-B inhibitors among the compounds in the series, with **46** exhibiting >1300 times more selectivity for hMAO-B over hMAO-A inhibition. Interestingly, the 3,4-dimethylbenzene and 3-chloro-4-fluorobenzene derivatives (**45** and **50**) were potent but nonselective hMAO-B inhibitors.

Our studies with compound **1** (detailed in the Biology section within the Experimental Section) suggest that it behaves as an irreversible inhibitor to the hMAO enzyme.

In Vitro Neuroprotective Action in the PC12 Assay. Cell-based cell survival assays are relatively simplified systems and, therefore, widely used for in vitro high-throughput screening of potential anti-Parkinsonian drugs. We chose PC12 cells for this purpose, as they are catecholaminergic in origin, similar to dopaminergic neurons, and we used different types of neurotoxins to increase the predictive value of the model for clinical neuroprotection. In the first series of experiments, 6-hydroxydopamine (6-OHDA), a catecholaminergic neurotoxin, was used to elicit cell death, whereas in the second series, rotenone, a mitochondrial toxin implicated in the etiology of PD, was used. In these experiments, rasagiline was used as reference compound, and the procedures described by Zheng et al.[24] were followed, with slight modification. Cells were incubated with 6-OHDA (200 μM) for 24 h, and test compounds and rasagiline were applied in a single 10^{-5} M concentration for 1 h prior to 6-OHDA application. The MTT assay was used to assess cell death. In a similar manner, rotenone was used to assess the ability of the target compounds

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Table 3. MAO-A and -B Inhibition Values of All Group 1−4 Compounds Determined in the Human Recombinant Assay

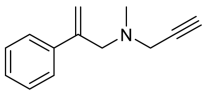
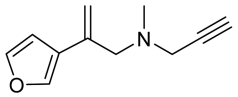
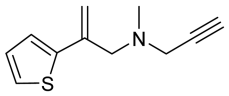
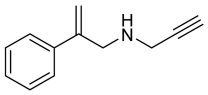
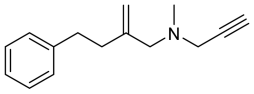
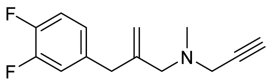
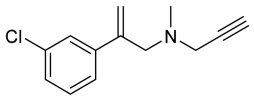
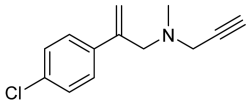
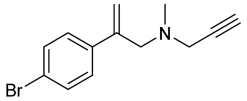
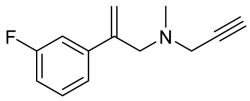
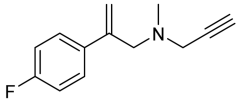
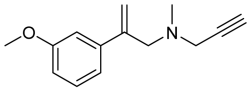
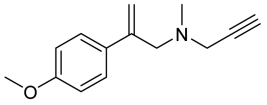
Compound		hMAO-B, IC_{50}, nM	hMAO-A, IC_{50}, nM	Selectivity, (A/B)
	GROUP 1			
1		60	3500	58
6		0.72	1900	2638
7		3.4	2500	735
	GROUP 2			
10		1500	20000	13
15		140	39	0.28
16		34	130	4
	GROUP 3			
19		30	83	3
20		3.9	120	31
22		0.85	60	70
24		7.6	160	21
25		3.7	280	74
27		65	200	3
28		370	56	0

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Table 3. continued

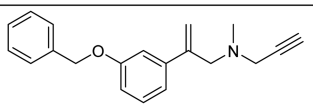
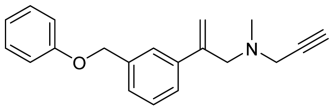
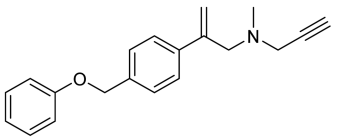
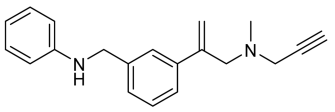
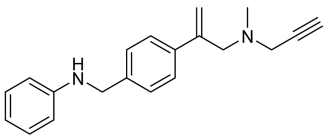
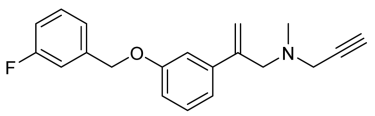
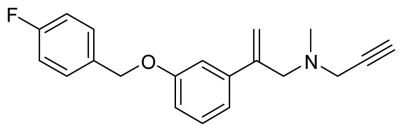
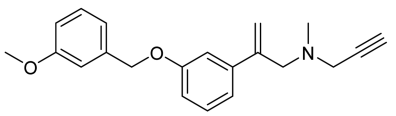
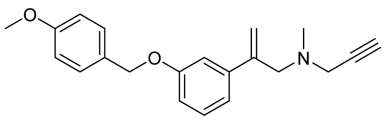
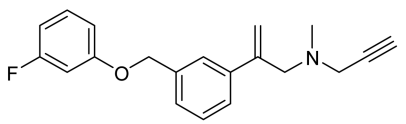
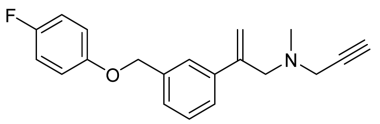
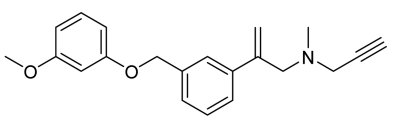
Compound		hMAO-B, IC_{50}, nM	hMAO-A, IC_{50}, nM	Selectivity, (A/B)
29		6	2100	350
31		20	400	20
32		50	620	12
33		7.4	1400	185
34		80	850	11
35		50	1200	24
36		13	63	5
37		6.6	590	88
38		50	2500	50
39		14	71	5
40		1.4	5.5	4
41		20	350	17

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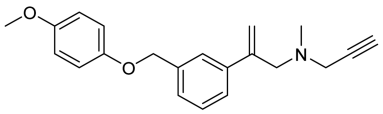
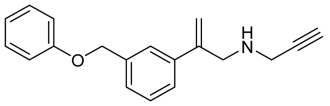
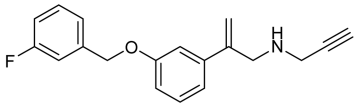
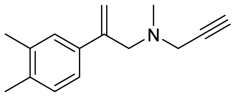
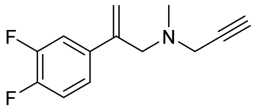
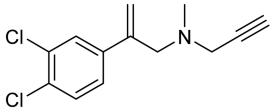
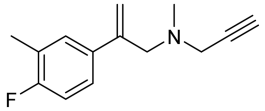
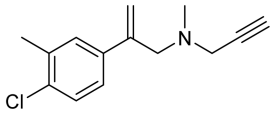
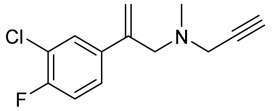
Table 3. continued

Compound		hMAO-B, IC_{50}, nM	hMAO-A, IC_{50}, nM	Selectivity, (A/B)
42		9	440	48
43		6.3	250	40
44		1.9	4200	2211
	GROUP 4			
45		18	91	5
46		2.3	3100	1347
47		1.0	61	61
48		17	230	14
49		4.1	100	24
50		6.3	26	4

to facilitate cell survival.[25] The results are summarized in Table 4.

In line with literature data,[24,26] rasagiline exerted significant, 48% protective effect in 10 μM concentration (Table 4). Of the compounds tested, compounds **1**, **10**, **15**, **18**, **20**, **24**, **31**, **46**, and **48** are protective against 6-OHDA-induced cell death (>40% protection), while only compounds **15**, **21**, and **45** show >20% protection against rotenone-induced cell death. Of these, compound **24** showed the highest increase of 117% in the survival of 6-OHDA-treated cells, while the best compound for rotenone-induced cell death is less spectacular at 33% increase (shown by compound **15**). The latter is, however, also a very important finding, since **15** is still much more effective than rasagiline (6% increase) on rotenone treatment of PC-12 cells. It is interesting to note that, among those compounds which were tested in both assays, compound **15**, a selective hMAO-A inhibitor, is the only compound that shows neuroprotective activities in both 6-OHDA- and rotenone-treated PC12 cells.

Cytotoxicity of Selected Compounds and Their Putative Metabolites. The cytotoxicities of selected compounds (**1**, **20**, **40**, **43**, and **46**) and their principal metabolites (**a**–**c**, Scheme 4) were also investigated using a metabolically competent immortalized liver cell line, TAMH (TGFα overexpressing mouse hepatocytes). This assay provides a glimpse of the safety index of the compounds, i.e., the margin of activity vs toxicity, to support further development. Viability at 100 and 10 μM (with 24 and 72 h exposures) was used as a preliminary indicator of cytotoxicity.

From Table 5, it is noted that rasagiline and its metabolite, aminoindane, along with compounds **1**, **20**, and **46** and their

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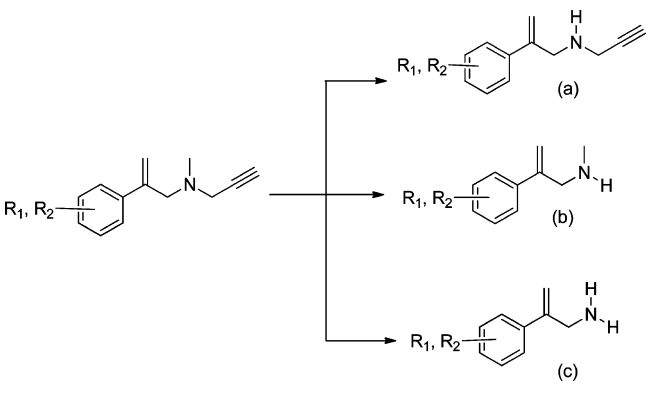
Table 4. Effect of Compounds (10 μM) on the Survival of PC12 Cells after 6-OHDA and Rotenone Treatment[a]

compd	% survival	
	6-OHDA	rotenone
control (no test compd)	100 ± 2.31	100 ± 1.88
rasagiline	147.82 ± 7.23***	106.05 ± 1.28
1	166.71 ± 7.17***	116.8 ± 2.1
2	N.D.	121.01 ± 4.20
3	N.D.	106.05 ± 3.15
4	N.D.	110 ± 4.15
5	N.D.	113.11 ± 2.35
6	132.9 ± 7.3	115.7 ± 4.2
7	127.94 ± 8.87	104.57 ± 7.3
9	N.D.	115.10 ± 3.0
10	151.781 ± 8.79***	111.68 ± 4.6
11	110.61 ± 1.59	97.45 ± 3.24
12	108.95 ± 2.13	100.79 ± 1.91
14	102.83 ± 1.00	100.05 ± 5.39
15	163.99 ± 9.7***	133.40 ± 2.4***
17	110.14 ± 1.69	113.06 ± 7.0
18	167.06 ± 11.74***	101.86 ± 1.99
19	108.81 ± 5.335	107.92 ± 3.54
20	147.17 ± 6.76**	112.03 ± 4.8
21	110.24 ± 3.52	131.64 ± 6.76***
22	119.23 ± 2.62	106.25 ± 5.03
23	117.26 ± 2.95	109.17 ± 4.6
24	217.27 ± 14.92***	101.92 ± 3.24
25	119.89 ± 4.81	102.12 ± 1.2
26	105.03 ± 1.52	111.07 ± 5.3
27	112.02 ± 3.56	109.19 ± 1.17
28	115.66 ± 2.52	109.72 ± 4.6
29	95.39 ± 7.89	89.67 ± 2.18
31	174.24 ± 6.27***	106.90 ± 0.99
44	109.50 ± 1.94	109.51 ± 2.13
45	119.89 ± 2.16	125.96 ± 7.50*
46	174.46 ± 14.07**	114.48 ± 4.68
47	81.27 ± 6.87	89.31 ± 3.18
48	156.54 ± 9.13**	88.61 ± 5.12
49	106.09 ± 9.79	104.46 ± 4.16
50	111.49 ± 1.18	93.95 ± 2.65

[a]Survival data are expressed as the percentage of 6-OHDA- and rotenone-treated cells. Symbols represent significant changes from 6-OHDA- and rotenone-treated (*P < 0.05; **P < 0.01; ***P < 0.001) cells, respectively. Statistical analysis: one-way ANOVA followed by the Tukey test. All data are the means ± SEM of at least eight values measured in two independent plates. N.D. not determined.

Scheme 4. Principal Metabolites of Propargylamines



respective metabolites are generally not toxic, even at supra-pharmacological concentrations (100 μM) after 24 and 72 h, respectively. However, for compound **46**, high toxicities were observed after 72 h at both 100 and 10 μM. For compounds **40** and **40a**, cell viability is less than 50% when administered at 100 μM concentration after 24 h. This loss of cell viability is more prominent for **40** and all its metabolites at the concentration of 100 μM. Similar observations were also made for the metabolites of **31**, with cell viabilities ranging between 15 and 40% after 72 h with treatment of 100 μM of test compound. These compounds with measurable toxicities prompted IC_{50} determination, which revealed that **40** is the most toxic (28.3 μM) followed by the desmethyl metabolite of **31** (i.e., **43**, IC_{50} = 33.7 μM) that is followed by the desmethyl metabolite of **40**, i.e., **40a** (IC_{50} = 43.9 μM). Nonetheless, these studies show that most of the novel compounds and their metabolites can be considered to be non-cytotoxic at the projected concentrations used for MAO B inhibition and therapeutic effect.

Finally, ADMET properties of a selected set of compounds (**1**, **20**, **31**, **35**, **46**) were characterized by QikProp parameters (see the Supporting Information, Table 5) obtained with the Schrodinger Suite in silico modeling package. No violation of druglikeness scores was observed.

CONCLUSIONS

The studies above have identified a number of new compounds possessing selective and potent human MAO B inhibitory activities that also possess in vitro neuroprotective (as measured by survival of 6-OHDA- or rotenone-treated cells) properties. The most promising of these compounds are identified as compound **1**, the *m*-fluorophenyl compound **24**, the *m*-benzyloxyphenyl compound **31**, 3,4-dimethylphenyl compound **45**, 3,4-difluorophenyl compound **46**, and the 3-methyl-4-fluorophenyl analogue **48**. On the basis of these highly promising results, further studies on these compounds are now in progress to confirm their roles in the development of disease-modifying anti-Parkinsonian drugs.

EXPERIMENTAL SECTION

General. All reaction solvents were dried by the PureSolv MD 7 Solvent Purification System (Innovative Technology) prior to use. All reagents were used as purchased without further purification. All reactions were conducted under a slight positive pressure of argon using oven-dried (110 °C) glassware. Room temperature corresponds to a temperature interval from 20 to 25 °C. The solvents were removed under reduced pressure using standard rotary evaporators.

Analytical thin layer chromatography (TLC) separations were performed on Merck's silica gel 60 F_{254}-precoated aluminum sheets. Visualization was accomplished with UV light (254 or 365 nm) and/or ninhydrin (200.0 mmol in ethanol) stain followed by heating. Solvent mixtures used for chromatography are always given as a vol/vol ratio. Flash column chromatography was generally performed using silica gel 60 (spherical, 40−100 μm or 40−63 μm), purchased from Sigma-Aldrich, or the Biotage SP1 purification system by gradient. Melting points were determined on a Büchi-540 capillary melting point apparatus and are uncorrected. Fourier-transform infrared spectroscopy was conducted using Spectrum 100 software (PerkinElmer). High-resolution mass spectrometry was run by the electrospray ionization time-of-flight (ESI-TOF) mode on an Agilent 6210 mass spectrometer.

High-performance liquid chromatography (HPLC) analysis was performed with the following systems: (a) a JASCO PU-2089 pump and a JASCO MD-2010 diode array detector, with manual injection of 20 μL on a column kept at ambient temperature (25 °C) at a MP flow rate of 0.5 mL/min or (b) Shimadzu LC-20AD parallel double micro

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Table 5. Cytotoxicity of Selected Compounds/Principal Metabolites

	viability				
	24 h		72 h		
compd	100 μM	10 μM	100 μM	10 μM	IC_{50} (μM)
rasagilline mesylate	88.8 ± 1.7	90.5 ± 2.8	84.1 ± 1.7	91.6 ± 3.8	
aminoindane	87.5 ± 2.9	96.4 ± 1.7	81.2 ± 2.4	82.7 ± 1.6	
1	83.3 ± 4.1	76.6 ± 7.0	88.6 ± 1.7	92.6 ± 2.2	
10 (=**1a**)	85.2 ± 2.9	93.2 ± 3.3	71.9 ± 2.8	90.3 ± 3.4	
1b	93.3 ± 2.5	89.8 ± 8.4	95.0 ± 4.1	92.8 ± 7.7	
1c	80.9 ± 2.7	89.8 ± 2.7	78.3 ± 3.5	89.1 ± 4.4	
20	63.1 ± 3.5	72.7 ± 5.0	96.2 ± 4.7	94.0 ± 2.8	
20a	71.8 ± 2.5	80.9 ± 4.2	84.3 ± 1.1	96.6 ± 3.5	
20b	74.2 ± 6.0	75.6 ± 1.6	81.2 ± 4.3	86.0 ± 10.4	
20c	58.5 ± 10.9	71.7 ± 3.9	62.5 ± 6.4	67.5 ± 14.7	
31	66.9 ± 5.3	90.2 ± 4.5	71.3 ± 8.7	92.6 ± 8.6	
43 (=**31a**)	38.2 ± 11.2	84.1 ± 5.5	15.8 ± 5.4	90.3 ± 7.4	33.7
31b	65.6 ± 1.5	82.6 ± 9.2	39.4 ± 7.7	87.1 ± 3.9	80.7
31c	50.1 ± 6.9	78.8 ± 2.6	22.4 ± 5.9	83.0 ± 2.2	86.4
40	47.1 ± 8.7	70.6 ± 7.5	19.9 ± 19.8	59.1 ± 6.1	28.3
40a	31.8 ± 6.8	69.3 ± 2.3	6.5 ± 11.8	70.8 ± 10.6	43.9
40b	61.6 ± 7.2	89.8 ± 5.8	27.0 ± 9.9	87.1 ± 4.1	
40c	55.2 ± 4.8	89.6 ± 2.2	29.8 ± 8.4	66.4 ± 0.7	
46	107.3 ± 1.4	113.3 ± 9.4	3.95 ± 15.5	5.1 ± 25.4	
46a	82.3 ± 2.8	93.6 ± 4.5	77.1 ± 3.5	81.8 ± 0.9	
46b	103.6 ± 0.6	113.3 ± 3.5	83.1 ± 4.4	84.4 ± 1.0	
46c	110.3 ± 0.8	112.2 ± 1.6	79.2 ± 0.9	84.4 ± 1.5	

plunger type chromatograph, Shimadzu SPD-M20A Prominence HPLC photo diode array detector, and Shimadzu SIL-20A Prominence HPLC autosampler, with autoinjection of 10−30 μL, a column kept at 40 °C, and a MP flow rate of 1 mL/min. The following are the conditions for system a: condition A used a Phenomenex Gemini NX C18 250 × 4.6 mm, 5 μm analytical column and ACN/H_2O 65:35 as eluent, and condition B used a Phenomenex Gemini NX C18 250 × 4.6 mm, 5 μm analytical column and ACN/H_2O 80:20 as eluent. For system b, condition C used a Kinetex 2.6 μm C18 100 Å, LC column 150 × 4.6 mm analytical column, with the following gradient: 35%−95% solvent B in gradient flow from 0 to 10 min, followed by 2 min of 95% solvent B and 3 min of 35% solvent B, where solvent A is water and solvent B is ACN spiked with 0.1% triethylamine.

Tandem liquid chromatography−mass spectrometry (LCMS) was performed on a Waters 2996 separations module and 3100 mass detector using a Luna-5u-C18(2) (150 × 4.6 mm) reverse-phase analytical column at a flow rate of 1.0 mL/min. The eluting system, consisting of A (water) and B (acetonitrile), was used under a linear gradient. The performance was monitored by photodiode array detection with a wavelength of 254 nm. Generally, the eluting program started at 80% of A and 20% of B for the first 2 min, followed by changing the concentration of B from 20% to 80% in 20 min. This condition was maintained for another 5 min.

Elemental analyses were performed on an Elementar VarioEL III apparatus. Microwave (mw) irradiation experiments were carried out in a monomode CEM-Discover mw reactor, using the standard configuration as delivered, including proprietary software. The experiments were executed in open vessel mode with control of the temperature by infrared detection. After completion of the reaction, the vial was cooled to 50 °C by air jet cooling.

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature (if not indicated otherwise), in the solvent indicated, on a Bruker 400 Ultra Shield spectrometer operating at 400 and 101 MHz, a Varian Mercury Plus 400 spectrometer at frequency of 400 and 100 MHz, a Varian Unity 600 spectrometer at frequency at 600 and 150 MHz, or on a Bruker Avance III 500 spectrometer at frequency of 500 or 125 MHz, respectively. Chemical shifts for ^1H NMR were recorded in parts per million (ppm) downfield from tetramethylsilane or the proton signal of residual nondeuterated solvent (δ 7.26 for $CHCl_3$, δ 4.87 for CH_3OH, δ 4.79 for H_2O, δ 2.50 for DMSO, and δ 2.05 for acetone) as the internal signal. Coupling constants are indicated in hertz (Hz). Chemical shifts are given using the δ-scale relative to tetramethylsilane or the residual solvent signal as an internal reference. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet doublet, dm = doublet multiplet, and tm = triplet multiplet.

Chemistry. Detailed experimental procedures for the syntheses of the base form of the target compounds and the respective intermediates are described in the Supporting Information. The data below are for the final compounds used in the biological studies.

General Synthetic Procedure for Salt Formation from the Free Base (Method A). To a solution of the appropriate base in 96% EtOH or dioxane or EtOAc, 37% HCl was added dropwise or HCl gas was bubbled into an ice-cooled solution of the amine in ether until pH <3. After 30 min of stirring at room temperature, the residue was filtered or the mixture was evaporated to dryness. The residue obtained was recrystallized to afford the salts in 15−93% yields (unoptimized), depending on the scale.

General Synthetic Procedure for Salt Formation from the Free Base (Method B). A stock solution of oxalic acid (2.6 mmol, MW = 126.07, oxalic acid dihydrate) in abs *i*-PrOH (5.0 mL) was prepared and used immediately. Stock solutions should not be kept since ester formation has previously been observed. To a solution of the appropriate base (0.9 mmol) in abs *i*-PrOH (0.5 mL) was added a 0.512 M solution of oxalic acid dihydrate in *i*-PrOH (1.8 mL, 0.9 mmol). The resulting solution was allowed to stir at rt for 30 min, and then it was kept at 0 °C for 10 min. Then, the solid was filtered, washed with ether (2.0 mL), and dried under high vacuum to afford the products in 50−95% unoptimized yields.

N-Methyl-2-phenyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (1). Following method A, the title compound was isolated (87%): mp 166−167 °C; ^1H NMR (400 MHz, D_2O) δ 7.58−7.44 (m, 5H), 5.84 (s, 1H), 5.70 (br s, 1H), 4.42 (br s, 2H), 4.01 (d, J = 2.4 Hz, 2H), 3.17 (t, J = 2.4 Hz, 1H), 2.90 (s, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 140.3, 139.2, 132.0, 131.9, 129.2, 127.0, 83.1, 74.0, 61.3, 47.6,

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42.4. Anal. Calcd for $C_{12}H_{15}N$·HCl (MW = 221.73): C, 70.42%; H, 7.27%; N, 6.32%. Found: C, 70.50%; H, 7.51%; N, 6.30%.

*N-Methyl-N-(prop-2-yn-1-yl)-2-(pyridin-2-yl)prop-2-en-1-aminium Carboxyformate (**2**).* Following method B, the title compound was isolated (60%): mp 113−114 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (ddd, J = 4.8, 1.6, 0.8 Hz, 1H), 7.78 (td, J = 8.0, 1.6 Hz, 1H), 7.69 (dt, J = 8.0, 0.8 Hz, 1H), 7.30 (ddd, J = 8.0, 4.8, 1.2 Hz, 1H), 6.09 (d, J = 1.6 Hz, 1H), 5.50 (d, J = 0.4 Hz, 1H), 3.67 (s, 2H), 3.47 (d, J = 2.0 Hz, 2H), 3.35 (t, J = 2.0 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.7, 155.8, 148.7, 142.2, 136.8, 122.8, 120.8, 119.4, 77.6, 77.2, 57.8, 44.7, 40.7; HRMS (ESI+) m/z calcd for $C_{12}H_{14}N_2^+$ $[M + H]^+$ 187.1230, found 187.1230. Anal. Calcd for $C_{12}H_{14}N_2$·$(COOH)_2$ (MW = 276.29): C, 60.86%; H, 5.84%; N, 10.14%. Found: C, 59.90%; H, 5.88%; N, 10.10%.

*3-(3-(Methyl(prop-2-yn-1-yl)ammonio)prop-1-en-2-yl)pyridin-1-ium Carboxyformate (**3**).* Following method B, the title compound was isolated (55%): mp 149−150 °C; ^{1}H NMR (DMSO-d_6) δ 8.74 (dd, J = 2.0, 0.4 Hz, 1H), 8.48 (dd, J = 4.8, 1.6 Hz, 1H), 7.93 (ddd, J = 8.0, 2.0, 1.6 Hz, 1H), 7.37 (ddd, J = 8.0, 4.8, 0.8 Hz, 1H), 5.69 (d, 1H, J = 0.8 Hz), 5.40 (d, 1H, J = 0.8 Hz), 3.55 (s, 2H), 3.39 (d, 2H, J = 2.4 Hz), 3.29 (t, 1H, J = 2.4 Hz), 2.25 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 161.2, 148.4, 147.3, 141.1, 134.4, 133.9, 123.3, 117.9, 78.1, 76.8, 58.9, 44.3, 40.5; HRMS (ESI+) m/z calcd for $C_{12}H_{14}N_2^+$ $[M + H]^+$ 187.1230, found 187.1229. Anal. Calcd for $C_{12}H_{14}N_2$·2$(COOH)_2$ (MW = 366.32): C, 52.46%; H, 4.95%; N, 7.65%. Found: C, 51.86%; H, 5.04%; N, 7.64%.

*4-(3-(Methyl(prop-2-yn-1-yl)ammonio)prop-1-en-2-yl)pyridin-1-ium Carboxyformate (**4**).* Following method B, the title compound was isolated (50%): mp 158−160 °C; ^{1}H NMR (DMSO-d_6) δ 8.53 (d, J = 6.0 Hz, 1H), 7.54 (d, J = 6.0 Hz, 1H), 5.80 (s, 1H), 5.45 (s, 1H), 3.46 (s, 2H), 3.32 (d, J = 2.4 Hz, 2H), 3.23 (t, J = 2.4 Hz, 1H), 2.19 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 161.3, 149.4, 146.4, 141.9, 121.1, 119.4, 78.3, 76.5, 58.5, 44.3, 40.6; HRMS (ESI+) m/z calcd for $C_{12}H_{14}N_2^+$ $[M + H]^+$ 187.1230, found 187.1227. Anal. Calcd for $C_{12}H_{14}N_2$·2$(COOH)_2$ (MW = 366.32): C, 52.46%; H, 4.95%; N, 7.65%. Found: C, 51.18%; H, 5.13%; N, 7.68%.

*2-(Furan-2-yl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**5**).* Following method B, the title compound was isolated (50%): mp 121−122 °C; ^{1}H NMR (DMSO-d_6) δ 7.63 (dd, J = 2.0, 0.8 Hz, 1H), 6.59 (dd, J = 3.2, 0.8 Hz, 1H), 6.49 (dd, J = 3.2, 2.0 Hz, 1H), 5.60 (d, J = 1.2 Hz, 1H), 5.18 (d, J = 1.2 Hz, 1H), 3.42 (d, J = 2.4 Hz, 2H), 3.35 (s, 2H), 3.27 (t, J = 2.4 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 161.5, 152.7, 142.7, 133.3, 112.2, 111.5, 107.9, 78.2, 76.8, 57.4, 44.6, 40.8; HRMS (ESI+) m/z calcd for $C_{11}H_{14}NO^+$ $[M + H]^+$ 176.1070, found 176.1069. Anal. Calcd for $C_{11}H_{13}NO$·$(COOH)_2$ (MW = 265.26): C, 58.86%; H, 5.70%; N, 5.28%. Found: C, 58.44%; H, 5.88%; N, 5.22%.

*2-(Furan-3-yl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**6**).* Following method B, the title compound was isolated (77%): mp 116−118 °C; ^{1}H NMR (DMSO-d_6) δ 7.84 (s, 1H), 7.61 (t, J = 1.6 Hz, 1H), 6.74 (dd, J = 1.6, 0.8 Hz, 1H), 5.46 (s, 1H), 5.12 (s, 1H), 3.38 (s, 2H), 3.30 (s, 2H), 3.25 (s, 1H), 2.26 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 161.3, 143.3, 140.2, 135.4, 124.7, 113.9, 108.5, 78.2, 76.6, 59.3, 44.5, 40.7; HRMS (ESI+) m/z calcd for $C_{11}H_{14}NO^+$ $[M + H]^+$ 176.1070, found 176.1072. Anal. Calcd for $C_{11}H_{13}NO$·$(COOH)_2$ (MW = 265.26): C, 58.86%; H, 5.70%; N, 5.28%. Found: C, 57.84%; H, 5.81%; N, 5.26%.

*N-Methyl-N-(prop-2-yn-1-yl)-2-(thiophen-2-yl)prop-2-en-1-amine Hydrochloride (**7**).* Following method A, the title compound was isolated (36%): mp 155 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (br s, 1H), 7.55 (m, 1H), 7.39 (m, 1H), 7.10 (dd, J = 5.1, 3.6 Hz, 1H), 5.80 (br s, 1H), 5.60 (br s, 1H), 4.16 (br s, 2H), 4.08 (br s, 2H), 3.87 (br s, 1H), 2.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 141.4, 131.8, 128.0, 126.7, 126.1, 120.3, 81.5, 73.3, 56.3, 45.3, 44.4. Anal. Calcd for $C_{11}H_{13}NS$·HCl (MW = 227.75): C, 58.01%; H, 6.20%; N, 6.15%. Found: C, 57.98%; H, 6.22%; N, 5.97%.

*N-Methyl-N-(prop-2-yn-1-yl)-2-(thiophen-3-yl)prop-2-en-1-aminium Carboxyformate (**8**).* Following method B, the title compound was isolated (55%): mp 125−126 °C; ^{1}H NMR (DMSO-d_6) δ 7.78 (dd, J = 2.8, 1.2 Hz, 1H), 7.64 (dd, J = 4.8, 2.8 Hz, 1H), 7.52 (dd, J = 4.8, 1.2 Hz, 1H), 5.74 (s, 1H), 5.34 (s, 1H), 3.55 (bs, 4H), 3.40 (s, 1H), 2.41 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 161.3, 141.3, 140.3, 126.1, 125.9, 122.0, 115.0, 78.0, 76.9, 59.5, 44.5, 40.7; HRMS (ESI+) m/z calcd for $C_{11}H_{14}NS^+$ $[M + H]^+$ 192.0841, found 192.0844. Purity by HPLC (condition A) 100%.

*N-Methyl-N-(prop-2-yn-1-yl)-2-(thiazol-2-yl)prop-2-en-1-aminium Carboxyformate (**9**).* Following method B, the title compound was isolated (60%): mp 153−154 °C; ^{1}H NMR (DMSO-d_6) δ 7.82 (d, J = 3.2 Hz, 1H), 7.69 (d, J = 3.2 Hz, 1H), 6.04 (s, 1H), 5.47 (s, 1H), 3.49 (s, 2H), 3.41 (d, J = 2.4 Hz, 2H), 3.25 (t, J = 2.4 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.1, 161.4, 142.7, 137.7, 120.7, 118.5, 78.1, 76.7, 58.3, 44.7, 40.7; HRMS (ESI+) m/z calcd for $C_{10}H_{13}N_2S^+$ $[M + H]^+$ 193.0794, found 193.0795. Purity by HPLC (condition A) 97%.

*2-Phenyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (**10**).* Following method A, the title compound was isolated (14%): mp 135 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.56−7.43 (m, 5H), 5.77 (br s, 1H), 5.59 (br s, 1H), 4.29 (br s, 2H), 3.93 (d, J = 2.6 Hz, 2H), 3.03 (t, J = 2.6 Hz, 1H). Anal. Calcd for $C_{12}H_{13}N$·HCl (MW = 207.70): C, 69.39%; H, 6.97%; N, 6.74%. Found: C, 69.10%; H, 6.80%; N, 6.73%.

*N-Ethyl-2-phenyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**11**).* To the amine (89.6 mg, 0.449 mmol) in *i*-PrOH (0.39 mL) was added a solution of oxalic acid (40.4 mg, 0.448 mmol) in ^{i}PrOH (0.39 mL). A white solid did not form, even after the reaction was left at 0 °C overnight. The oil was left stirring in ether for 3 d. Eventually, a white solid which formed was filtered, washed with cold ether, and dried to give the title compound (86.8 mg, 67%): mp 85−87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53−7.51 (m, 2H), 7.35−7.26 (m, 3H), 5.52 (d, J = 1.2 Hz, 1H), 5.29 (d, J = 1.2 Hz, 1H), 3.53 (s, 2H), 3.38 (d, J = 2.4 Hz, 2H), 3.17 (t, J = 2.4 Hz, 1H), 2.57 (q, J = 7.2 Hz, 2H), 1.00 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.8, 144.3, 139.6, 128.6, 128.1, 126.7, 116.5, 78.5, 76.8, 57.5, 47.1, 40.9, 12.5; HRMS (ESI+) m/z calcd for $C_{16}H_{19}NNaO_4^+$ $[M + Na]^+$ 312.1206, found 312.1202. Purity by HPLC (condition C) 99%.

*N-Methyl-N-(2-phenylallyl)but-3-yn-2-aminium Carboxyformate (**12**).* Following method B, the title compound was isolated as a white solid (63%): mp 108.5−110.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52−7.50 (m, 2H), 7.35−7.28 (m, 3H), 5.50 (d, J = 1.2 Hz, 1H), 5.26 (d, J = 1.2 Hz, 1H), 3.67 (qd, J = 6.8, 2.0 Hz, 1H), 3.55 (d, J = 13.6 Hz, 1H), 3.35 (d, J = 13.6 Hz, 1H), 3.23 (d, J = 2.0 Hz, 1H), 2.14 (s, 3H), 1.23 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 144.1, 139.3, 128.1, 127.6, 126.2, 115.8, 81.7, 75.8, 58.2, 49.7, 36.5, 19.2; HRMS (ESI+) m/z calcd for $C_{14}H_{18}N^+$ $[M + H]^+$ 200.1434, found 200.1430. Purity by HPLC (condition A) 99%.

*(rac)-N-Methyl-3-phenyl-N-(prop-2-ynyl)but-3-en-2-amine (**13**).* This was submitted as a free base. Please refer to the Supporting Information for the method to synthesize the free base. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.42 (m, 2H), 7.26−7.10 (m, 3H), 5.25−5.23 (m, 2H), 3.46 (q, J = 6.8 Hz, 1H), 3.42 (t, J = 2.4 Hz, 2H), 2.30 (s, 3H), 2.16 (t, J = 2.4 Hz, 1H), 1.12 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.6, 141.1, 128.0, 127.3, 127.1, 114.5, 79.2, 73.0, 61.9, 43.4, 39.1, 18.1. EI MS: m/z (relative abundance) 200 ($M^{+\bullet}$, 25%); 131 (100%). Purity by HPLC (conditions C) 95%.

*N-Methyl-4-phenyl-N-(prop-2-yn-1-yl)pent-4-en-1-aminium Carboxyformate (**14**).* Following method B, the title compound was isolated (77%): mp 96−97 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.48−7.45 (m, 2H), 7.36−7.26 (m, 3H), 5.33 (d, J = 1.2 Hz, 1H), 5.14 (d, J = 1.2 Hz, 1H), 3.83 (d, J = 2.4 Hz, 2H), 3.04 (t, J = 2.4 Hz, 1H), 3.00 (t, J = 7.6 Hz, 2H), 2.67 (s, 3H), 2.63 (t, J = 7.6 Hz, 2H), 1.83 (m, 2H); ^{13}C NMR (101 MHz, acetone-d_6) δ 163.3, 148.2, 141.5, 129.3, 128.4, 126.9, 113.2, 79.0, 55.1, 45.1, 40.5, 32.9, 24.2; HRMS (ESI+) m/z calcd for $C_{15}H_{20}N^+$ $[M + H]^+$ 214.1590, found 214.1596. Anal. Calcd for $C_{15}H_{19}N$·$(COOH)_2$ (MW = 303.36): C, 67.31%; H, 6.98%; N, 4.62%. Found: C, 66.62%; H, 6.83%; N, 4.54%.

*N-Methyl-2-methylene-4-phenyl-N-(prop-2-yn-1-yl)butan-1-aminium Carboxyformate (**15**).* Following method B, the title compound was isolated as a white solid (38%): mp 116−118 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29−7.25 (m, 2H), 7.22−7.14 (m, 3H), 4.96 (s,

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1H), 4.93 (s, 1H), 3.36 (s, 2H), 3.23 (s, 1H), 3.05 (s, 2H), 2.72 (t, J = 8.0 Hz, 2H), 2.32 (t, J = 8.0 Hz, 2H), 2.24 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.4, 144.9, 141.7, 128.19, 128.16, 125.68, 113.66, 78.18, 76.56, 60.33, 44.64, 40.75, 35.11, 33.17; HRMS (ESI+) m/z calcd for $C_{15}H_{20}N^+$ $[M + H]^+$ 214.1596, found 214.1595. Anal. Calcd for $C_{15}H_{19}N\cdot(COOH)_2$ (MW = 303.35): C, 67.31%; H, 6.98%; N, 4.62%. Found: C, 67.38%; H, 6.80%; N, 4.59%.

*2-(3,4-Difluorobenzyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**16**).* Following method B, the title compound was isolated as a white solid (78%): mp 98−99 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.23−7.12 (m, 2H), 7.05−7.01 (m, 1H), 5.35 (d, J = 1.2 Hz, 1H), 5.19 (d, J = 1.2 Hz, 1H), 3.86 (d, J = 2.4 Hz, 2H), 3.51 (s, 2H), 3.46 (s, 2H), 3.12 (t, J = 2.4 Hz, 1H), 2.73 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.8, 151.5 (dd, J = 248.4, 12.8 Hz), 150.5 (dd, J = 248.4, 12.8 Hz), 142.0, 137.0 (dd, J = 5.7, 4.0 Hz), 126.7 (dd, J = 6.3, 3.5 Hz), 121.4, 119.0 (d, J = 17.3 Hz), 118.3 (d, J = 17.2 Hz), 79.5, 74.8, 60.2, 45.9, 41.0, 40.6 (d, J = 1.4 Hz); HRMS (ESI+) m/z calcd for $C_{14}H_{16}F_2N^+$ $[M + H]^+$ 236.1245, found 236.1249. Anal. Calcd for $C_{14}H_{15}F_2N\cdot(COOH)_2$ (MW = 325.31): C, 59.07%; H, 5.27%; N, 4.31%. Found: C, 59.02%; H, 5.31%; N, 4.15%.

*N-Methyl-2-phenyl-N-(prop-2-en-1-yl)prop-2-en-1-amine Hydrochloride (**17**).*[27] Following method A, the title compound was isolated (64%): mp 127−128 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (br s, 1H), 7.55 (m, 2H), 7.44−7.34 (m, 3H), 6.05 (m, 1H), 5.83 (br s, 1H), 5.81 (br s, 1H), 5.51−5.43 (m, 2H), 4.30 (m, 1H) and 4.16 (m, 1H), 3.78 (m, 1H) and 3.76 (m, 1H), 2.60 (br s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 141.7, 138.1, 128.7, 128.5, 127.6, 126.3, 124.8, 122.7, 57.3, 56.5, 38.8. Anal. Calcd for $C_{13}H_{17}N\cdot HCl$ (MW = 223.74): C, 69.79%; H, 8.11%; N, 6.26%. Found: C, 69.54%; H, 8.22%; N, 6.40%.

*(E)-3-Fluoro-N-methyl-2-phenyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**18**).* Following method B, the title compound was isolated as a white solid (67%): mp 153−155 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, J = 7.6 Hz, 2H), 7.36 (t, J = 7.6 Hz, 2H), 7.28 (d, J = 7.6 Hz, 1H), 7.03 (d, J = 84.0 Hz, 1H), 3.32 (d, J = 2.0 Hz, 2H), 3.28 (d, J = 3.2 Hz, 2H), 3.20 (brs, 1H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 147.3 (d, J = 265.3 Hz), 133.7, 128.1 (d, J = 4.5 Hz), 128.1, 127.5, 119.0, 78.3, 76.5, 54.5 (d, J = 9.2 Hz), 44.3, 40.3; HRMS (ESI+) m/z calcd for $C_{13}H_{15}FN^+$ $[M + H]^+$ 204.1183, found 204.1184. Purity by HPLC (condition A) >99%.

*2-(3-Chlorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**19**).* Following method B, the title compound was isolated (78%): mp 139−140 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.58 (t, J = 2.0 Hz, 1H), 7.50 (dt, J = 6.8, 2.0 Hz, 1H), 7.39−7.33 (m, 2H), 5.60 (d, J = 1.2 Hz, 1H), 5.31 (d, J = 1.2 Hz, 1H), 3.45 (s, 2H), 3.33 (d, J = 2.0 Hz, 2H), 3.21 (t, J = 2.0 Hz, 1H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 142.5, 141.3, 133.0, 130.0, 127.4, 126.1, 125.0, 117.6, 78.2, 76.5, 59.1, 44.3; HRMS (ESI+) m/z calcd for $C_{13}H_{15}ClN^+$ $[M + H]^+$ 220.0888, found 220.0888. Anal. Calcd for $C_{13}H_{14}ClN\cdot(COOH)_2$ (MW = 309.74): C, 58.16%; H, 5.21%; N, 4.52%; Found: C, 57.88%; H, 5.15%; N, 4.43%.

*2-(4-Chlorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**20**).* Following method B, the title compound was isolated (88%): mp 160−161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (m, 2H), 7.38 (m, 2H), 5.57 (d, J = 1.1 Hz, 1H), 5.31 (q, J = 0.9 Hz, 1H), 3.50 (br s, 2H), 3.37 (d, J = 2.4 Hz, 2H), 3.25 (t, J = 2.4 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6, 142.3, 137.7, 132.4, 128.2, 128.2, 117.4, 77.9, 76.9, 59.0, 44.3, 40.6. Anal. Calcd for $C_{13}H_{14}ClN\cdot(COOH)_2$ (MW = 309.74): C, 58.16%; H, 5.21%; N, 4.52%. Found: C, 58.04%; H, 5.13%; N, 4.46%.

*2-(3-Bromophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**21**).* Following method B, the title compound was isolated as a white solid (56%): mp 130−132 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (s, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 5.59 (s, 1H), 5.31 (s, 1H), 3.45 (s, 2H), 3.34 (d, J = 2.0 Hz, 2H), 3.21 (t, J = 2.0 Hz, 1H), 2.22 (br, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.3, 142.5, 141.5, 130.3, 130.3, 128.9, 125.4, 121.7, 117.7, 78.2, 76.5, 59.1, 44.3, 40.6; HRMS (ESI+) m/z calcd for $C_{13}H_{15}BrN^+$ $[M + H]^+$ 264.0382, found 264.0381. Anal. Calcd for $C_{13}H_{14}BrN\cdot(COOH)_2$ (MW = 354.20): C, 50.86%; H, 4.55%; N, 3.95%. Found: C, 50.48%; H, 4.51%; N, 3.90%.

*2-(4-Bromophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (**22**).* Following method A, the title compound was isolated (49%): mp 176−177 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 11.10 (br s, 1H), 7.61−7.51 (m, 4H), 5.85 (br s, 1H), 5.78 (br s, 1H), 4.24 (br s, 2H), 4.03 (br s, 2H), 3.86 (br s, 1H), 2.69 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.0, 136.8, 131.4, 128.6, 123.8, 121.8, 81.4, 73.2, 56.4, 44.1, 39.2. Anal. Calcd for $C_{13}H_{14}BrN\cdot HCl$ (MW = 300.62): C, 51.94%; H, 5.03%; N, 4.66%. Found: C, 51.84%; H, 5.11%; N, 4.52%.

*2-(2-Fluorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (**23**).* Following method A, the title compound was isolated (73%): mp 140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (m, 1H), 7.42 (m, 1H), 7.27−7.21 (m, 2H), 6.00 (br s, 1H), 5.72 (br s, 1H), 4.22 (br s, 2H), 4.15 (br s, 2H), 3.84 (br s, 1H), 2.70 (br s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.2 (d, J = 243 Hz), 133.7, 130.5 (d, J = 8 Hz), 130.2 (d, J = 3 Hz), 126.9, 126.2 (d, J = 13 Hz), 124.8 (d, J = 3 Hz), 115.9 (d, J = 22 Hz), 81.4, 73.0, 57.2, 44.1, 39.0. Anal. Calcd for $C_{13}H_{14}FN\cdot HCl$ (MW = 239.71): C, 65.13%; H, 6.31%; N, 5.84%. Found: C, 65.09%; H, 6.18%; N, 5.78%.

*2-(3-Fluorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**24**).* Following method B, the title compound was isolated (60%): mp 142.2−142.9 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.45−7.40 (m, 1H), 7.37−7.30 (m, 2H), 7.14−7.09 (m, 1H), 5.81 (s, 1H), 5.68 (s, 1H), 4.22 (s, 2H), 3.94 (d, J = 2.5 Hz, 2H), 3.26 (t, J = 2.5 Hz, 1H), 2.79 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.6, 164.5 (d, J = 245.2 Hz), 141.4 (d, J = 7.7 Hz), 140.1 (d, J = 2.4 Hz), 131.8 (d, J = 8.4 Hz), 124.5, 123.6 (d, J = 2.9 Hz), 116.6 (d, J = 21.4 Hz), 114.6 (d, J = 22.7 Hz), 80.6, 73.9, 59.4, 45.9, 40.8. Anal. Calcd for $C_{13}H_{14}FN\cdot(COOH)_2$ (MW = 293.29): C, 61.43%; H, 5.50%; N, 4.78%. Found: C, 61.42%; H, 5.43%; N, 4.64%.

*2-(4-Fluorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (**25**).* Following method A, the title compound was isolated (62%): mp 182−183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (br s, 1H), 7.60 (m, 2H), 7.21 (m, 2H), 5.77 (br s, 1H), 5.74 (br s, 1H), 4.36−4.16 (br m, 2H), 4.02 (br s, 2H), 3.85 (br s, 1H), 2.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.0 (d, J = 245 Hz), 137.8, 134.9 (d, J = 3 Hz), 129.4 (d, J = 8 Hz), 123.8, 116.1 (d, J = 22 Hz), 82.2, 73.9, 57.3, 44.8, 40.0. Anal. Calcd for $C_{13}H_{14}FN\cdot HCl$ (MW = 239.72): C, 65.13%; H, 6.31%; N, 5.84%. Found: C, 65.26%; H, 6.11%; N, 5.84%.

*2-(2-Methoxyphenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (**26**).* Following method A, the title compound was isolated (44%): mp 135 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (br s, 1H), 7.36 (ddd, J = 8.0, 7.6, 1.8 Hz, 1H), 7.27 (dd, J = 7.6, 1.8 Hz, 1H), 7.04 (dm, J = 8.0 Hz, 1H), 6.97 (td, J = 7.2, 1.0 Hz, 1H), 5.82 (br s, 1H), 5.52 (br s, 1H), 4.36−4.05 (m, 2H), 4.00 (br s, 2H), 3.85 (br s, 1H), 3.81 (s, 3H), 2.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.3, 137.7, 129.9, 129.9, 127.5, 124.6, 120.7, 111.4, 81.2, 73.1, 57.2, 55.4, 44.1, 39.1. Anal. Calcd for $C_{14}H_{17}NO\cdot HCl$ (MW = 251.75): C, 66.79%; H, 7.21%; N, 5.56%. Found: C, 66.70%; H, 7.11%; N, 5.49%.

*2-(3-Methoxyphenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**27**).* Following method B, the title compound was isolated as a white solid (86%): mp 106−107 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (t, J = 8.0 Hz, 1H), 7.12−7.08 (m, 1H), 7.06 (t, J = 2.4 Hz, 1H), 6.87 (ddd, J = 8.0, 2.4, 0.4 Hz, 1H), 5.56 (d, J = 1.2 Hz, 1H), 5.29 (d, J = 1.2 Hz, 1H), 3.76 (s, 3H), 3.53 (s, 2H), 3.41 (d, J = 2.4 Hz, 2H), 3.27 (t, J = 2.4 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7, 159.2, 143.1, 140.4, 129.2, 118.6, 117.0, 113.1, 112.1, 77.8, 77.0, 59.1, 55.0, 44.4, 40.6; HRMS (ESI+) m/z calcd for $C_{14}H_{18}NO^+$ $[M + H]^+$ 216.1383, found 216.1391. Anal. Calcd for $C_{14}H_{17}NO\cdot(COOH)_2$ (MW = 305.33): C, 62.94%; H, 6.27%; N, 4.59%. Found: C, 62.87%; H, 6.14%; N, 4.56%.

*2-(4-Methoxyphenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-amine Hydrochloride (**28**).* Following method A, the title compound was isolated (93%): mp 148−149 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (m, 2H), 6.96 (m, 2H), 5.71 (br s, 1H), 5.62 (br s, 1H), 4.23 (br s, 2H), 4.04 (br s, 2H), 3.88 (br s, 1H), 3.77 (s, 3H), 2.70 (s,

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3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.6, 137.4, 129.8, 127.7, 121.0, 114.0, 81.5, 73.2, 56.7, 55.2, 44.2, 39.3. Anal. Calcd for $C_{14}H_{17}NO{\cdot}HCl$ (MW = 251.75): C, 66.79%; H, 7.21%; N, 5.56%. Found: C, 66.36%; H, 7.09%; N, 5.51%.

*2-(3-(Benzyloxy)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**29**).* Following method B, the title compound was isolated (73%): mp 112−113 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.48−7.31 (m, 6H), 7.16−7.15 (m, 1H), 7.12−7.09 (m, 1H), 7.03 (ddd, J = 8.0, 2.4, 0.8 Hz, 1H), 5.75 (d, J = 0.8 Hz, 1H), 5.61 (d, J = 0.8 Hz, 1H), 5.13 (s, 2H), 4.22 (s, 2H), 3.92 (d, J = 2.4 Hz, 2H), 3.27 (t, J = 2.4 Hz, 1H), 2.77 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.9, 160.7, 140.8, 140.2, 138.6, 131.2, 129.5, 129.0, 128.6, 123.7, 120.2, 116.5, 114.5, 80.7, 73.7, 71.1, 59.6, 45.9, 40.7; HRMS (ESI+) m/z calcd for $C_{20}H_{22}NO^+$ $[M + H]^+$ 292.1696, found 292.1689. Anal. Calcd for $C_{20}H_{21}NO{\cdot}(COOH)_2$ (MW = 381.42): C, 69.28%; H, 6.08%; N, 3.67%. Found: C, 69.25%; H, 6.07%; N, 3.52%.

*2-(4-(Benzyloxy)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**30**).* Following method B, the title compound was isolated (66%): mp 138−139 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49−7.30 (m, 7H), 6.97 (d, J = 8.8 Hz, 2H), 5.45 (d, J = 1.2 Hz, 1H), 5.16 (d, J = 0.4 Hz, 1H), 5.11 (s, 2H), 3.46 (s, 2H), 3.37 (d, J = 2.4 Hz, 2H), 3.24 (t, J = 2.4 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.4, 158.0, 142.7, 137.1, 131.4, 128.4, 127.8, 127.6, 127.4, 114.6, 114.4, 78.0, 76.7, 69.2, 59.3, 44.3, 40.6; HRMS (ESI+) m/z calcd for $C_{20}H_{22}NO^+$ $[M + H]^+$ 292.1696, found 292.1697. Purity by HPLC (condition A) >99%.

*N-Methyl-2-(3-(phenoxymethyl)phenyl)-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**31**).* Following method B, the title compound was isolated as a white solid (85%): mp 125−126 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (br, 1H), 7.50−7.47 (m, 1H), 7.37−7.35 (m, 2H), 7.31−7.27 (m, 2H), 7.03−7.00 (m, 2H), 6.96−6.92 (m, 1H), 5.55 (d, J = 1.2 Hz, 1H), 5.29 (d, J = 1.2 Hz, 1H), 5.10 (s, 2H), 3.50 (s, 2H), 3.37 (d, J = 2.3 Hz, 2H), 3.23 (t, J = 2.3 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.3, 158.3, 143.6, 139.2, 137.0, 129.5, 128.3, 127.0, 125.7, 125.6, 120.7, 116.3, 114.8, 78.2, 76.5, 69.1, 59.2, 44.4, 40.7. Anal. Calcd for $C_{20}H_{21}NO{\cdot}(COOH)_2$ (MW = 381.42): C, 69.28%; H, 6.08%; N, 3.67%. Found: C, 69.21%; H, 5.72%; N, 3.74%.

*N-Methyl-2-(4-(phenoxymethyl)phenyl)-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**32**).* Following method B, the title compound was isolated as a white solid (75%): mp 150−151 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.32−7.26 (m, 2H), 7.00 (dd, J = 8.7, 0.9 Hz, 2H), 6.93 (t, J = 7.3 Hz, 1H), 5.55 (d, J = 1.6 Hz, 1H), 5.27 (d, J = 1.6 Hz, 1H), 5.09 (s, 2H), 3.49 (s, 2H), 3.36 (d, J = 2.3 Hz, 2H), 3.23 (t, J = 2.3 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.3, 158.3, 143.3, 138.5, 136.5, 129.5, 127.5, 126.3, 120.7, 116.3, 114.7, 78.1, 76.6, 68.7, 59.2, 44.3, 40.6; HRMS (ESI+) m/z calcd for $C_{20}H_{22}NO^+$ $[M + H]^+$ 292.1696, found 292.1691. Anal. Calcd for $C_{20}H_{21}NO{\cdot}(COOH)_2$ (MW = 381.42): C, 69.28%; H, 6.08%; N, 3.67%. Found: C, 68.52%; H, 6.16%; N, 3.29%.

*N-Methyl-2-(3-((phenylamino)methyl)phenyl)-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**33**).* Following method B, the title compound was isolated (80%): mp 152−154 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.54 (s, 1H), 7.45−7.33 (m, 3H), 7.09 (dd, J = 8.4, 7.6 Hz, 2H), 6.73−6.59 (m, 3H), 5.77 (s, 1H), 5.63 (s, 1H), 4.38 (s, 2H), 4.28 (s, 2H), 3.94 (d, J = 2.4 Hz, 2H), 3.29 (t, J = 2.4 Hz, 1H), 2.78 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.0, 149.4, 142.4, 140.5, 138.7, 130.3, 130.1, 129.3, 126.7, 126.2, 124.1, 118.7, 114.7, 81.2, 73.2, 59.5, 46.0, 40.7; HRMS (ESI+) m/z calcd for $C_{20}H_{23}N_2^+$ $[M + H]^+$ 291.1856, found 291.1861. Purity by HPLC (condition C) 96%.

*N-Methyl-2-(4-((phenylamino)methyl)phenyl)-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**34**).* Following method B, the title compound was isolated (85%): mp 139−140 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.49 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.06 (dd, J = 8.8, 7.2 Hz, 2H), 6.64−6.55 (m, 3H), 5.74 (s, 1H), 5.58 (s, 1H), 4.35 (s, 2H), 4.21 (s, 2H), 3.91 (d, J = 2.4 Hz, 2H), 3.26 (t, J = 2.4 Hz, 1H), 2.76 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.8, 149.7, 142.9, 140.8, 137.2, 130.0, 129.0, 127.7, 122.9, 118.2, 114.2, 80.6, 73.8, 59.7, 48.3, 45.9, 40.8; HRMS (ESI+) m/z calcd for $C_{20}H_{23}N_2^+$ $[M + H]^+$ 291.1856, found 291.1856. Purity by HPLC (condition C) 97%.

*2-(3-((3-Fluorobenzyl)oxy)phenyl)-N-methyl-N-(prop-2-yn-1-yl)-prop-2-en-1-aminium Carboxyformate (**35**).* Following method B, the title compound was isolated as a white solid (87%): mp 107−109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47−7.41 (m, 1H), 7.31−7.23 (m, 3H), 7.18−7.10 (m, 3H), 6.95−6.93 (m, 1H), 5.53 (d, J = 1.6 Hz, 1H), 5.26 (d, J = 1.6 Hz, 1H), 5.14 (s, 2H), 3.45 (s, 2H), 3.34 (d, J = 2.4 Hz, 2H), 3.22 (t, J = 2.4 Hz, 1H), 2.22 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.2 (d, J = 243.6 Hz), 161.3, 158.0, 143.5, 140.7, 140.1 (d, J = 7.4 Hz), 130.4 (d, J = 8.3 Hz), 129.2, 123.5 (d, J = 2.7 Hz), 119.0, 116.5, 114.5 (d, J = 20.9 Hz), 114.2 (d, J = 21.8 Hz), 113.9, 112.9, 78.2, 76.6, 68.3 (d, J = 1.7 Hz), 59.3, 44.4, 40.6. Anal. Calcd for $C_{20}H_{20}FNO{\cdot}(COOH)_2$ (MW = 399.41): C, 66.16%; H, 5.55%; N, 3.51%. Found: C, 65.97%; H, 5.21%; N, 3.59%.

*2-(3-((4-Fluorobenzyl)oxy)phenyl)-N-methyl-N-(prop-2-yn-1-yl)-prop-2-en-1-aminium Carboxyformate (**36**).* Following method B, the title compound was isolated as a white solid (68%): mp 117.6−118.9 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.50−7.45 (m, 2H), 7.34 (t, J = 8.0 Hz, 1H), 7.15 (t, J = 1.8 Hz, 1H), 7.13−7.07 (m, 3H), 7.04−7.01 (m, 1H), 5.75 (s, 1H), 5.61 (s, 1H), 5.11 (s, 2H), 4.20 (s, 2H), 3.91 (d, J = 2.8 Hz, 2H), 3.27 (t, J = 2.8 Hz, 1H), 2.76 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.8, 163.9 (d, J = 244.7 Hz), 160.6, 141.0, 140.3, 134.6 (d, J = 3.1 Hz), 131.2, 130.7 (d, J = 8.3 Hz), 123.4, 120.3, 116.4, 116.2 (d, J = 21.7 Hz), 114.5, 80.5, 73.9, 70.4, 59.7, 45.9, 40.8; HRMS (ESI+) m/z calcd for $C_{20}H_{21}FNO^+$ $[M + H]^+$ 310.1602, found 310.1589. Anal. Calcd for $C_{20}H_{20}FNO{\cdot}(COOH)_2$ (MW = 399.41): C, 66.16%; H, 5.55%; N, 3.76%. Found: C, 66.16%; H, 5.31%; N, 3.61%.

*2-(3-((3-Methoxybenzyl)oxy)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**37**).* Following method B, the title compound was isolated as a white solid (68%): mp 113.9−114.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (app t, 1H), 7.24 (app t, 1H), 7.16−7.15 (m, 1H), 7.11−7.09 (m, 1H), 7.03−7.01 (m, 2H), 6.94−6.92 (m, 1H), 6.90−6.88 (m, 1H), 5.52 (d, J = 1.2 Hz, 1H), 5.25 (d, J = 1.2 Hz, 1H), 5.08 (s, 2H), 3.76 (s, 3H), 3.44 (s, 2H), 3.34 (d, J = 2.4 Hz, 2H), 3.21 (t, J = 2.4 Hz, 1H), 2.22 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.3, 159.3, 158.2, 143.6, 140.6, 138.7, 129.5, 129.2, 119.8, 118.8, 116.4, 113.9, 113.2, 113.0, 78.3, 76.5, 69.0, 59.3, 55.0, 44.4, 40.7. Anal. Calcd for $C_{21}H_{23}NO_2{\cdot}(COOH)_2$ (MW = 411.45): C, 67.14%; H, 6.12%; N, 3.40%. Found: C, 66.98%; H, 6.21%; N, 3.07%.

*2-(3-((4-Methoxybenzyl)oxy)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**38**).* Following method B, the title compound was isolated as a white solid (57%): mp 104−105 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.38−7.31 (m, 3H), 7.13 (s, 1H), 7.10 (d, J = 7.7 Hz, 1H), 7.02 (dd, J = 8.2, 2.1 Hz, 1H), 6.94−6.91 (m, 2H), 5.74 (s, 1H), 5.60 (br d, 1H), 5.05 (s, 2H), 4.19 (br, 2H), 3.90 (br, 2H), 3.80 (s, 3H), 3.26 (t, J = 2.4 Hz, 1H), 2.75 (s, 3H); ^{13}C NMR (100 MHz, methanol-d_4) δ 165.8, 161.0, 160.7, 141.4, 140.3, 131.2, 130.5, 130.4, 122.9, 120.1, 116.4, 114.9, 114.5, 80.2, 74.2, 70.9, 59.9, 55.7, 45.9, 40.9; HRMS (ESI+) m/z calcd for $C_{21}H_{24}NO_2^+$ $[M + H]^+$ 322.1802, found 322.1801. Anal. Calcd for $C_{21}H_{23}NO_2{\cdot}(COOH)_2$ (MW = 411.45): C, 67.14%; H, 6.12%; N, 3.40%. Found: C, 66.96%; H, 5.89%; N, 3.39%.

*2-(3-((3-Fluorophenoxy)methyl)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**39**).* Following method B, the title compound was isolated as a white solid (65%): mp 126.1−127.0 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.62 (br, 1H), 7.52−7.43 (m, 3H), 7.30−7.24 (m, 1H), 6.84−6.81 (m, 1H), 6.77 (dt, J = 11.1, 2.4 Hz, 1H), 6.70−6.65 (m, 1H), 5.78 (d, J = 0.8 Hz, 1H), 5.63 (d, J = 0.8 Hz, 1H), 5.13 (s, 2H), 4.23 (s, 2H), 3.91 (d, J = 2.8 Hz, 2H), 3.24 (t, J = 2.8 Hz, 1H), 2.77 (s, 3H); ^{13}C NMR (100 MHz, methanol-d_4) δ 164.8 (d, J = 183.0 Hz), 161.6 (d, J = 11.0 Hz), 141.3, 139.3, 139.2, 131.6 (d, J = 10.1 Hz), 130.3, 129.1, 127.3, 126.8, 123.2, 112.0 (d, J = 3.0 Hz), 108.6 (d, J = 21.5 Hz), 103.5 (d, J = 25.2 Hz), 80.2, 74.2, 71.0, 59.8, 46.0, 40.9; HRMS (ESI+) m/z calcd for $C_{20}H_{21}FNO^+$ $[M + H]^+$ 310.1602, found 310.1599. Anal. Calcd for

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$C_{20}H_{20}FNO\cdot(COOH)_2$ (MW = 399.41): C, 66.16%; H, 5.55%; N, 3.51%. Found: C, 66.05%; H, 5.35%; N, 3.61%.

*2-(3-((4-Fluorophenoxy)methyl)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**40**).* Following method B, the title compound was isolated as a white solid 59%): mp 111.0−112.2 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.61 (brs, 1H), 7.51−7.42 (m, 3H), 7.02−6.97 (m, 4H), 5.76 (s, 1H), 5.61 (s, 1H), 5.10 (s, 2H), 4.19 (s, 2H), 3.88 (s, 2H), 3.22 (s, 1H), 2.74 (s, 3H); ^{13}C NMR (100 MHz, methanol-d_4) δ 165.6, 158.8 (d, J = 237.2 Hz), 156.3 (d, J = 2.1 Hz), 141.3, 139.6, 139.2, 130.3, 129.1, 127.2, 126.8, 123.2, 117.2 (d, J = 8.0 Hz), 116.7 (d, J = 23.4 Hz), 80.2, 74.2, 71.4, 59.8, 45.9, 40.9; HRMS (ESI+) m/z calcd for $C_{20}H_{21}FNO^+$ $[M + H]^+$ 310.1602, found 310.1599. Anal. Calcd for $C_{20}H_{20}NO\cdot(COOH)_2$ (MW = 399.41): C, 66.16%; H, 5.55%; N, 3.51%. Found: C, 65.66%; H, 5.59%; N, 3.56%.

*2-(3-((3-Methoxyphenoxy)methyl)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**41**).* Following method B, the title compound was isolated as a white solid (55%): mp 114.7−116.0 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.62 (s, 1H), 7.52−7.41 (m, 3H), 7.16 (t, J = 8.0 Hz, 1H), 6.60−6.51 (m, 3H), 5.77 (s, 1H), 5.62 (s, 1H), 5.11 (s, 2H), 4.21 (s, 2H), 3.89 (s, 2H), 3.76 (s, 3H), 3.23 (s, 1H), 2.75 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 165.6, 162.4, 161.3, 141.3, 139.7, 139.2, 131.0, 130.3, 129.1, 127.1, 126.7, 123.2, 108.3, 107.5, 102.5, 80.3, 74.2, 70.7, 59.8, 55.7, 45.9, 40.9; HRMS (ESI+) m/z calcd for $C_{21}H_{24}NO_2^+$ $[M + H]^+$ 322.1802, found 322.1805. Anal. Calcd for $C_{21}H_{23}NO_2\cdot(COOH)_2$ (MW = 411.45): C, 67.14%; H, 6.12%; N, 3.40%. Found: C, 67.16%; H, 5.90%; N, 3.53%.

*2-(3-((4-Methoxyphenoxy)methyl)phenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**42**).* Following method B, the title compound was isolated as a white solid (47%): mp 117.8−180 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (brs, 1H), 7.49−7.46 (m, 1H), 7.35−7.34 (m, 2H), 6.97−6.93 (m, 2H), 6.88−6.83 (m, 2H), 5.53 (d, J = 1.2 Hz, 1H), 5.28 (d, J = 1.2 Hz, 1H), 5.03 (s, 2H), 3.69 (s, 3H), 3.47 (s, 2H), 3.35 (d, J = 2.4 Hz, 2H), 3.21 (t, J = 2.4 Hz, 1H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 153.5, 152.3, 143.6, 139.2, 137.2, 128.3, 127.0, 125.6, 125.5, 116.2, 115.8, 114.6, 78.3, 76.5, 69.7, 59.2, 55.3, 44.4, 40.7; HRMS (ESI+) m/z calcd for $C_{21}H_{24}NO_2^+$ $[M + H]^+$ 322.1802, found 322.1804. Purity by HPLC (condition B) 99%.

*2-(3-(Phenoxymethyl)phenyl)-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**43**).* Following method B, the title compound was isolated as a white solid (81%): mp 188−190 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.60 (s, 1H), 7.50−7.43 (m, 3H), 7.27 (dd, J = 8.8, 7.6 Hz, 2H), 6.99 (dd, J = 8.8, 0.8 Hz, 2H), 6.94 (m, 1H), 5.75 (s, 1H), 5.55 (s, 1H), 5.13 (s, 2H), 4.23 (d, J = 0.4 Hz, 2H), 3.91 (d, J = 2.4 Hz, 2H), 3.21 (t, J = 2.4 Hz, 1H); ^{13}C NMR (101 MHz, methanol-d_4) δ 166.4, 160.1, 141.1, 139.8, 138.8, 130.5, 130.3, 129.2, 126.8, 126.5, 122.1, 120.5, 116.0, 79.3, 74.8, 70.6, 50.7, 37.2; HRMS (ESI+) m/z calcd for $C_{19}H_{20}NO^+$ $[M + H]^+$ 278.1539, found 278.1542. Purity by HPLC (condition C) 99%.

*2-(3-((3-Fluorobenzyl)oxy)phenyl)-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**44**).* Following method B, the title compound was isolated as a white solid (84%): mp 182−184 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.42−7.33 (m, 2H), 7.27−7.25 (m, 1H), 7.22−7.19 (m, 1H), 7.14−7.13 (m, 1H), 7.12−7.09 (m, 1H), 7.07−7.02 (m, 2H), 5.72 (s, 1H), 5.53 (s, 1H), 5.16 (s, 2H), 4.19 (s, 2H), 3.90 (d, J = 2.4 Hz, 2H), 3.22 (t, J = 2.4 Hz, 1H); ^{13}C NMR (101 MHz, methanol-d_4) δ 166.5, 164.4 (d, J = 244.6 Hz), 160.4, 141.5 (d, J = 7.3 Hz), 141.1, 140.1, 131.4 (d, J = 8.2 Hz), 131.2, 124.1 (d, J = 2.9 Hz), 120.4, 120.1, 116.3, 115.5 (d, J = 21.4 Hz), 115.0 (d, J = 22.3 Hz), 114.4, 79.2, 74.8, 70.2 (d, J = 2.0 Hz), 50.8, 37.2; HRMS (ESI+) m/z calcd for $C_{19}H_{19}FNO^+$ $[M + H]^+$ 296.1445, found 296.1460. Anal. Calcd for $C_{19}H_{18}FNO\cdot(COOH)_2$ (MW = 385.39): C, 65.45%; H, 5.23%; N, 3.63%. Found: C, 65.21%; H, 5.06%; N, 3.82%.

*2-(3,4-Dimethylphenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**45**).* Following method B, the title compound was isolated (71%): mp 136−137 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.30 (s, 1H), 7.24 (dd, J = 7.6, 2.0 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 5.71 (d, J = 0.4 Hz, 1H), 5.55 (d, J = 0.4 Hz, 1H), 4.24 (s, 2H), 3.94 (d, J = 2.4 Hz, 2H), 3.26 (s, 1H), 2.80 (s, 3H), 2.30 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 166.0, 140.7, 138.9, 138.4, 136.1, 131.3, 128.7, 125.1, 122.5, 80.9, 73.6, 59.6, 45.9, 40.7, 19.9, 19.5. Anal. Calcd for $C_{15}H_{19}N\cdot(COOH)_2$ (MW = 303.35): C, 67.31%; H, 6.98%; N, 4.62%. Found: C, 67.32%; H, 6.93%; N, 4.30%.

*2-(3,4-Difluorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**46**).* Following method B, the title compound was isolated as a white solid (60%): mp 147−148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66−7.56 (m, 1H), 7.38 (m, 2H), 5.59 (s, 1H), 5.30 (s, 1H), 3.44 (s, 2H), 3.34 (d, J = 2.1 Hz, 2H), 3.21 (t, J = 2.1 Hz, 1H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 150.2, 147.8, 141.7, 136.7, 123.2 (dd, J = 3.3, 6.3 Hz), 117.5, 117.1 (d, J = 17.0 Hz), 115.4 (d, J = 17.7 Hz), 78.2, 76.5, 59.2, 44.3, 40.5; HRMS (ESI+) m/z calcd for $C_{13}H_{13}F_2NNa^+$ $[M + Na]^+$ 244.0908, found 244.0913. Anal. Calcd for $C_{13}H_{13}F_2N\cdot(COOH)_2$ (MW = 311.28): C, 57.88%; H, 4.86%; N, 4.50%. Found: C, 57.38%; H, 4.82%; N, 4.40%.

*2-(3,4-Dichlorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**47**).* Following method B, the title compound was isolated as a white solid (81%): mp 139−140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 2.0 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.53 (dd, J = 8.4, 2.0 Hz, 1H), 5.65 (d, J = 0.8 Hz, 1H), 5.34 (d, J = 0.8 Hz, 1H), 3.45 (s, 2H), 3.33 (d, J = 2.4 Hz, 2H), 3.22 (t, J = 2.4 Hz, 1H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 141.6, 139.7, 130.9, 130.2, 130.1, 128.2, 126.6, 118.2, 78.2, 76.5, 59.0, 44.3, 40.5; HRMS (ESI+) m/z calcd for $C_{13}H_{14}Cl_2N^+$ $[M + H]^+$ 254.0498, found 254.0492. Anal. Calcd for $C_{13}H_{13}Cl_2N\cdot(COOH)_2$ (MW = 344.19): C, 52.34%; H, 4.39%; N, 4.07%. Found: C, 53.12%; H, 4.44%; N, 4.12%.

*2-(4-Fluoro-3-methylphenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**48**).* Following method B, the title compound was isolated as a white solid (76%): mp 158−159 °C; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.51−7.28 (m, 2H), 7.05 (app t, 1H), 5.71 (s, 1H), 5.60 (s, 1H), 4.23 (s, 2H), 3.96 (s, 2H), 3.28 (s, 1H), 2.81 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (101 MHz, methanol-d_4) δ 164.5, 161.7 (d, J = 246.0 Hz), 138.6, 133.3 (d, J = 3.5 Hz), 129.7 (d, J = 5.2 Hz), 125.7 (d, J = 8.3 Hz), 125.1 (d, J = 17.6 Hz), 122.0, 115.0 (d, J = 22.9 Hz), 79.4, 72.2, 58.2, 44.5, 39.3, 13.1 (d, J = 3.5 Hz). Anal. Calcd for $C_{14}H_{16}FN\cdot(COOH)_2$ (MW = 307.32): C, 62.53%; H, 5.90%; N, 4.56%. Found: C, 62.53%; H, 6.15%; N, 4.34%.

*2-(4-Chloro-3-methylphenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**49**).* Following method B, the title compound was isolated as a white solid (73%): mp 171−173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (br, 1H), 7.36 (br, 2H), 5.54 (d, J = 1.2 Hz, 1H), 5.27 (d, J = 1.2 Hz, 1H), 3.45 (s, 2H), 3.34 (d, J = 2.4 Hz, 2H), 3.22 (t, J = 2.4 Hz, 1H), 2.33 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.3, 142.7, 137.9, 135.0, 132.5, 129.0, 128.5, 125.5, 116.6, 78.2, 76.5, 59.1, 44.3, 40.6, 19.6; HRMS (ESI+) m/z calcd for $C_{14}H_{17}ClN^+$ $[M + H]^+$ 234.1044, found 234.1044. Anal. Calcd for $C_{14}H_{16}ClN\cdot(COOH)_2$ (MW = 323.77): C, 59.35%; H, 5.60%; N, 4.33%. Found: C, 60.07%; H, 5.60%; N, 4.25%.

*2-(3-Chloro-4-fluorophenyl)-N-methyl-N-(prop-2-yn-1-yl)prop-2-en-1-aminium Carboxyformate (**50**).* Following method B, the title compound was isolated as a white solid (77%): mp 144−145 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (dd, J = 7.2, 2.4 Hz, 1H), 7.57−7.53 (m, 1H), 7.37 (app t, 1H), 5.59 (d, J = 0.6 Hz, 1H), 5.30 (d, J = 0.6 Hz, 1H), 3.45 (s, 2H), 3.34 (d, J = 2.3 Hz, 2H), 3.22 (t, J = 2.3 Hz, 1H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 156.7 (d, J = 247.1 Hz), 141.6, 136.8 (d, J = 3.9 Hz), 128.3, 127.0 (d, J = 7.3 Hz), 119.2 (d, J = 17.6 Hz), 117.6, 116.5 (d, J = 20.8 Hz), 78.2, 76.5, 59.2, 44.3, 40.5. Anal. Calcd for $C_{13}H_{13}ClFN\cdot(COOH)_2$ (MW = 327.74): C, 54.97%; H, 4.61%; N, 4.27%. Found: C, 55.55%; H, 4.63%; N, 4.22%.

Biology. *Rat Monoamine Oxidase B and A Enzyme Assays.* MAO-A and MAO-B enzyme inhibitory activities were determined on rat brain homogenates with ^{14}C-5-HT (MAO-A) or ^{14}C-PEA (MAO-B) substrates and liquid scintillation method.

1. Tissue Preparation. MAO-A and MAO-B enzyme activities were determined on nuclei-free rat brain homogenates. Following

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decapitation, the brain (excluding cerebellum) was removed, the exact weight being determined. On 1.0 g of brain tissue was layered 2.0 mL of 0.25 M freshly dissolved saccharose. The sample was homogenized (blade homogenizer) in an ice−water bath (33.3% homogenate) and centrifuged (1000 rpm, 10 min, 4 °C). For the assays the diluted supernatants were used (MAO-B, 8.325%; MAO-A, 16.5%).

2. Radiometric Assay of MAO-B/MAO-A Enzyme Activity. MAO-B/MAO-A enzyme activity was determined by a radiometric method.[28] From ^{14}C-phenylethylamine/^{14}C-5-hydroxytriptamine substrate MAO-B/MAO-A forms ^{14}C-phenylacetaldehyde/^{14}C-5-hydroxyindole-3-acetaldehyde, which could be extracted under acidic conditions and quantitated. The incubation mixtures contained phosphate buffer (100 μL, 0.1 M, pH 7.4), EDTA (20 μL, 0.01 M), distilled (deionized) water/inhibitor (20 μL), and ^{14}C-phenylethylamine (MAO-B; 20 μL, 0.2 mM)/^{14}C-hydroxytriptamine (MAO-A, 20 μL, 0.5 mM), and 40 μL of tissue (nuclei-free homogenate). Mixtures were preincubated with the inhibitors for 10 min (37.5 °C), followed by subsequent addition of the substrate and incubation for 20 min at 37 °C. The reaction was stopped by adding 200 μL of 2 M citric acid. The samples were extracted with 1 mL of ethyl acetate by vortexing and then centrifuged (2500 rpm, 5 min). The enzyme activity was determined by liquid scintillation counting (600 μL of the organic phase + 5 mL of scintillation liquid).

Human Monoamine Oxidase B and A Enzyme Assays. MAO-A and MAO-B enzyme inhibitory activities were determined on brain human recombinant MAO-A and MAO-B enzymes, and results are summarized in Tables 3 and 4.

Human recombinant MAO-A and MAO-B were purchased in the form of supersomes (BD Biosciences) expressed in Sf9. The enzymatic activity was measured in a fluorescent coupled reaction. MAO-A and MAO-B oxidize their substrates (5-hydroxytryptamine and phenylethylamine respectively) to produce hydrogen peroxide, which produces the oxidized form of Amplex UltraRed (Invitrogen) that can be readily measured in a fluorimetric plate reader at 540/590 nm (excitation/emission). Measurements were conducted in a 384-well format in the final volume of 40 μL. The enzymes were preincubated on ice for 1 h prior to tests. The drug candidates were incubated for 10 min at room temperature with the enzyme, and then substrate was added to initialize the reaction at 30 °C. Fluorescence was read at 1 h of reaction and corrected with the value read before substrate addition. Dose−response curves were measured using at least seven dilution points with 5-fold dilution steps. Duplicate points were determined for each concentration. IC_{50} values were calculated from the remaining activity, and the graphs were fitted using Origin 5.0 software.

Irreversible Inhibition of Monoamine Oxidase B. To investigate whether compound **1** inhibited MAO-B reversibly or irreversibly, we designed an equilibrium dialysis experiment. First, we measured the dose−response curve of **1** for MAO-B inhibition. For the dialysis experiment, a mixture of the enzyme MAO-B and **1** was prepared, where the concentration of compound **1** was 10× higher than its IC_{50} value, and the inhibition (86%) was measured. Then, the enzyme−inhibitor mixture was dialyzed overnight against 1000× volume of buffer containing no inhibitor; the percentage of inhibition was determined on the next day. The inhibition we obtained was 99%, indicating that the inhibitor could not be washed out. In a control experiment under similar conditions except that there was no inhibitor present, the enzyme activity did not change, indicating that the enzyme was intact after the overnight dialysis.

As a validation, we performed another study in which known reversible (safinamide) and irreversible inhibitors (rasagiline) were used. It was found that extent of inhibition caused by the irreversible inhibitor did not change by dialysis, while the extent of inhibition achieved by the reversible inhibitor was significantly decreased after the dialysis, indicating that the enzyme−inhibitor complex formed reversibly and dissociation occurred during the dialysis.

In the next experiment, the influence of preincubation of the enzyme−inhibitor mixture was investigated. We found that the longer the preincubation period, the lower the virtual IC_{50} value of compound **1**. These results could be interpreted as follows. Compound **1** first binds to MAO B with a K_i value of 60 nM, and then it irreversibly inactivates the enzyme; preincubation for 90 min at room temperature results in a virtual IC_{50} of 50 pM.

From the above experiments, it could be concluded that **1** inhibited the MAO-B enzyme in an irreversible fashion.

Cell Culture. PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM 4500) supplemented with 10% horse serum, 5% fetal bovine serum (FBS), and L-glutamine. The cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C. For experiments assessing the effect of drugs on 6-OHDA/rotenone-induced cell death, PC12 cells were seeded on 96-well plates in a density of 10 000 cells/well. The data are expressed as the percentage of the survival of 6-OHDA/rotenone-treated cells of the same plate. Every measurement was performed at least in $n = 8$ number and repeated on at least two independent plates.

MTT Assay. Cell viability was assessed using the colorimetric reagent 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). A stock solution of the dye was prepared, filter-sterilized, and stored at −20 °C. After 6-OHDA/rotenone treatment in 96-well plates, 5 mg/mL MTT was added to each well, and the incubation was continued for another 4 h. The converted dye was solubilized with acidic isopropyl alcohol (0.04 M HCl in anhydrous isopropyl alcohol). Reduced MTT was measured at a wavelength of 570 nm.

Cytotoxicity Assay. Transforming growth factor-α overexpressing mouse hepatocytes (TAMH) were used as the metabolically competent immortalized cell line to access the cytoxicity of compounds arising from both the administered ones and the metabolites. These cells were maintained in accordance to established conditions.[29] TAMH cells were seeded at a cell density of 12 000 cells/well in a 96-well plate for the cytotoxicity assays. Cells were first tested at three different concentrations, 100, 10, and 1 μM, at both 24 and 72 h of incubation, as a preliminary determinant of toxicity. Compounds exhibiting >50% reduction in viability were subjected to subsequent IC_{50} determination. These assays were performed with the CellTiter-Glo cell viability assay (Promega Corp.) as per the manufacturer's instructions. Resultant luminescence readings (as an indication of intracellular ATP levels as a surrogate for viability) were measured with an integration time of 0.25 s on a Tecan Infinite M200 microplate reader.

■ ASSOCIATED CONTENT

Supporting Information

Tables summarizing the intermediates synthesized by routes A−D, detailed synthetic procedures and characterization data, and calculated QikProp parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

PD, Parkinson's disease; MAO, monoamine oxidase; rMAO, rat monoamine oxidase; hMAO, human monoamine oxidase; 6-OHDA, 6-hydroxydopamine; COMT, catechol-*O*-methyl transferase; X-Phos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazo-

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lium bromide; TAMH, transforming growth factor-α overexpressing mouse hepatocytes.

REFERENCES

(1) Le, W.; Jankovic, J.; Xie, W.; Kong, R.; Appel, S. H. (−)-Deprenyl protection of 1-methyl-4 phenylpyridium ion (MPP+)-induced apoptosis independent of MAO-B inhibition. *Neurosci. Lett.* **1997**, *224*, 197−200.

(2) Youdim, M. B. H.; Bakhle, Y. S. Monoamine oxidase: Isoforms and inhibitors in Parkinson's disease and depressive illness. *Br. J. Pharmacol.* **2006**, *147*, S287−S296.

(3) Magyar, K. l. n.; Moussa, B. H. Y.; Peter, D. The pharmacology of selegiline. *Int. Rev. Neurobiol.* **2011**, *100*, 65−84.

(4) Youdim, M. B. H.; Gross, A.; Finberg, J. P. M. Rasagiline [*N*-propargyl-1*R*(+)-aminoindan], a selective and potent inhibitor of mitochondrial monoamine oxidase B. *Br. J. Pharmacol.* **2001**, *132*, 500−506.

(5) Youdim, M. H.; Weinstock, M. Molecular basis of neuroprotective activities of rasagiline and the anti-Alzheimer drug TV3326 [*N*-propargyl-(3*R*) aminoindan-5-yl)-ethyl methyl carbamate]. *Cell. Mol. Neurobiol.* **2001**, *21*, 555−573.

(6) Am, O. B.; Amit, T.; Youdim, M. B. H. Contrasting neuroprotective and neurotoxic actions of respective metabolites of anti-Parkinson drugs rasagiline and selegiline. *Neurosci. Lett.* **2004**, *355*, 169−172.

(7) Bar-Am, O.; Yogev-Falach, M.; Amit, T.; Sagi, Y.; Youdim, M. B. H. Regulation of protein kinase C by the anti-Parkinson drug, MAO-B inhibitor, rasagiline and its derivatives, in vivo. *J. Neurochem.* **2004**, *89*, 1119−1125.

(8) Maruyama, W.; Akao, Y.; Carrillo, M. C.; Kitani, K.-i.; Youdium, M. B. H.; Naoi, M. Neuroprotection by propargylamines in Parkinson's disease: Suppression of apoptosis and induction of prosurvival genes. *Neurotoxicol. Teratol.* **2002**, *24*, 675−682.

(9) Weinreb, O.; Bar-Am, O.; Amit, T.; Chillag-Talmor, O.; Youdim, M. B. H. Neuroprotection via pro-survival protein kinase C isoforms associated with Bcl-2 family members. *FASEB J.* **2004**, *18*, 1471−1473.

(10) Youdim, M. B. H.; Amit, T.; Falach-Yogev, M.; Am, O. B.; Maruyama, W.; Naoi, M. The essentiality of Bcl-2, PKC and proteasome−ubiquitin complex activations in the neuroprotective−antiapoptotic action of the anti-Parkinson drug, rasagiline. *Biochem. Pharmacol.* **2003**, *66*, 1635−1641.

(11) Ahlskog, J. E.; Uitti, R. J. Rasagiline, Parkinson neuroprotection, and delayed-start trials: Still no satisfaction? *Neurology* **2010**, *74*, 1143−1148.

(12) (a) Makoto, N.; Wakako, M. Functional mechanism of neuroprotection by inhibitors of type B monoamine oxidase in Parkinson's disease. *Expert Rev. Neurother.* **2009**, *9*, 1233−1250. (b) Makoto, N.; Wakako, M. Monoamine oxidase inhibitors as neuroprotective agents in age-dependent neurodegenerative disorders. *Curr. Pharm. Des.* **2010**, *16*, 2799−2817.

(13) (a) Clemo, G. R.; Morgan, W. M.; Raper, R. The lupin alkaloids. Part XII. The synthesis of *dl*-lupinine and *dl*-isolupinine. *J. Chem. Soc.* **1937**, 965−969. (b) Dalla Croce, P.; Ferraccioli, R.; Ritieni, A. Selective α-chlorination of acetylpyrroles. *Synthesis* **1990**, 212−213.

(14) Lebel, H.; Guay, D.; Paquet, V.; Huard, K. Highly efficient synthesis of terminal alkenes from ketones. *Org. Lett.* **2004**, *6*, 3047−3050.

(15) Lebel, H.; Paquet, V. Rhodium-catalyzed methylenation of aldehydes. *J. Am. Chem. Soc.* **2004**, *126*, 320−328.

(16) Hori, T.; Sharpless, K. B. Selenium-catalyzed nonradical chlorination of olefins with *N*-chlorosuccinimide. *J. Org. Chem.* **1979**, *44*, 4204−4208.

(17) Yamanaka, M.; Arisawa, M.; Nishida, A.; Nakagawa, M. An intriguing effect of $Yb(OTf)_3$−TMSCl in the halogenation of 1,1-disubstituted alkenes by NXS: Selective synthesis of allyl halides. *Tetrahedron Lett.* **2002**, *43*, 2403−2406.

(18) Billingsley, K.; Buchwald, S. L. Highly efficient monophosphine-based catalyst for the palladium-catalyzed Suzuki−Miyaura reaction of heteroaryl halides and heteroaryl boronic acids and esters. *J. Am. Chem. Soc.* **2007**, *129*, 3358−3366.

(19) Biscoe, M. R.; Fors, B. P.; Buchwald, S. L. A new class of easily activated palladium precatalysts for facile C−N cross-coupling reactions and the low temperature oxidative addition of aryl chlorides. *J. Am. Chem. Soc.* **2008**, *130*, 6686−6687.

(20) Kinzel, T.; Zhang, Y.; Buchwald, S. L. A new palladium precatalyst allows for the fast Suzuki−Miyaura coupling reactions of unstable polyfluorophenyl and 2-heteroaryl boronic acids. *J. Am. Chem. Soc.* **2010**, *132*, 14073−14075.

(21) Duboudin, J. G.; Jousseaume, B.; Saux, A. Reactifs de grignard vinyliques γ fonctionnels: I. Reactivite des organomagnesiens vis-a-vis d'alcools α acetyleniques en presence d'halogenures cuivreux. *J. Organomet. Chem.* **1979**, *168*, 1−11.

(22) Baughman, T. W.; Sworen, J. C.; Wagener, K. B. The facile preparation of alkenyl metathesis synthons. *Tetrahedron* **2004**, *60*, 10943−10948.

(23) McDonald, I. A.; Lacoste, J. M.; Bey, P.; Palfreyman, M. G.; Zreika, M. Enzyme-activated irreversible inhibitors of monoamine oxidase: Phenylallylamine structure−activity relationships. *J. Med. Chem.* **1985**, *28*, 186−193.

(24) Zheng, H.; Gal, S.; Weiner, L. M.; Bar-Am, O.; Warshawsky, A.; Fridkin, M.; Youdim, M. B. H. Novel multifunctional neuroprotective iron chelator-monoamine oxidase inhibitor drugs for neurodegenerative diseases: In vitro studies on antioxidant activity, prevention of lipid peroxide formation and monoamine oxidase inhibition. *J. Neurochem.* **2005**, *95*, 68−78.

(25) Milusheva, E.; Baranyi, M.; Kormos, E.; Hracskó, Z.; Sylvester Vizi, E.; Sperlágh, B. The effect of antiparkinsonian drugs on oxidative stress induced pathological [^{3}H]dopamine efflux after in vitro rotenone exposure in rat striatal slices. *Neuropharmacology* **2010**, *58* (4−5), 816−825.

(26) Bar-Am, O.; Amit, T.; Youdim, M. B. H. Aminoindan and hydroxyaminoindan, metabolites of rasagiline and ladostigil, respectively, exert neuroprotective properties in vitro. *J. Neurochem.* **2007**, *103*, 500−508.

(27) Patanova, I. M.; Spryskov, A. A.; Belyaev, V. A.; Rodionova, N. M. Preparation of α-chloromethylstyrene-based secondary and tertiary amines. 3. *Izv. Vuz. Khim. Kh. Tekh.* **1975**, *18*, 865−868.

(28) Petres, D. A. V. Inhibition of serotonin biosynthesis by 6-halotryptophans in vivo. *Biochem. Pharmacol.* **1971**, *20*, 1413−1420.

(29) Wu, J. C.; Merlino, G.; Cveklova, K.; Mosinger, B., Jr.; Fausto, N. Autonomous growth in serum-free medium and production of hepatocellular carcinomas by differentiated hepatocyte lines that overexpress transforming growth factor alpha 1. *Cancer Res.* **1994**, *54*, 964−973.

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