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'Click' assembly of selective inhibitors for MAO-A

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ABSTRACT

In this Letter, an efficient strategy for the fast construction of 108 compounds library was developed using click chemistry. The fingerprint of inhibitory activity toward MAO-A/B against this library was obtained, and four hit compounds were identified as selective inhibitors toward MAO-A. Docking study was carried out to demonstrate the binding mode between **a9** and MAO-A/B, and the result reveals that **a9** localized in the 'aromatic cage' and oriented to establish π–π stacking interactions with Tyr407, Tyr444 and FAD in MAO-A rather than in MAO-B.

Monoamine oxidases (MAOs, EC 1.4.3.4) are flavin adenine dinucleotide (FAD)-containing enzymes localized in the outer mitochondrial membranes of neuronal, glial and other cells. The following two MAO isozymes: MAO-A and MAO-B, have been distinguished by substrate specificity, sensitivity to inhibitors, and amino acid sequences. MAO-A, preferentially oxidizing norepinephrine and serotonin, is selectively inhibited by nanomolar concentrations of clorgyline. MAO-B preferentially deaminates β-phenylethylamine and benzylamine, and is irreversibly inhibited by nanomolar concentrations of L-deprenyl.[1] MAO-B inhibitor (MAO-B-I) is used as a coadjuvant in the treatment of Parkinson's disease and Alzheimer's disease,[2] while MAO-A inhibitor (MAO-A-I) is a new antidepressant and anti-anxiety agent.[3] Hence, selective inhibitors for MAO-A or MAO-B may be useful therapeutic agents,[4] and the amount of MAO inhibitors (MAO-Is) was notably increased during the last decade.[5] However, a high rate of neuro degenerative diseases mutation still outpaces conventional drug discovery efforts. Rapid synthesis of diverse analogs of MAO-Is still remains a challenge.

Click chemistry, which is firstly developed by Sharpless, has enabled researchers to assemble two building blocks rapidly and efficiently on both laboratory and production scales.[6,7] The copper(I)-catalyzed 1,3-cycloaddition is an ideal tool for achieving diversity in just a few steps with satisfactory purity. Advantages of this reaction in biological studies have recently been demonstrated in several applications: construction of fluorescent oligonucleotides for DNA sequencing;[8] chemically orthogonal high fidelity bioconjugation;[9] and activity-based protein profiling in whole proteomes.[10] Moreover, due to its modularity, good selectivity, and wide scope, the copper(I)-catalyzed triazole formation has also been adopted widely for high-throughput discovery of enzyme inhibitors. Ashraf Brik et al. synthesized and screened HIV-1 PR inhibitors in the microtiter plate format.[11] Yao group developed small-molecule inhibitors targeting caspases.[12] On the other hand, we noticed that five-member ring heterocycles with two or four nitrogen atoms have been found as reversible and selective MAO-A/B inhibitors (Fig. 1).[13] We infer that 1,2,3-triazole derivates containing 3 nitrogen atoms may be a new type of inhibitor toward MAO-A/B. Herein, we report the 'click' synthesis of 1,4-disubstituted-1*H*-1,2,3-triazoles, and high-throughput screening of inhibitory activity toward MAO-A and B.

Firstly, R^1 building block azide compounds (**a–l**) and R^2 building block (**2**, **3**) were prepared according to the reported methods, while the other compounds were commercial available (Scheme 1).[14–16] Although Cu(I)-catalyzed 1,3-dipolar cycloaddition is a highly reliable process, which proceeds well in aqueous solvent and tolerates virtually all functional groups. It is still essential to investigate the minimum amount of catalysts used in the reaction owing to the sensitivity of MAO-A/B toward Cu(I or II) ions. Therefore, to eliminate interference arose from $CuSO_4$ and sodium ascorbate in microplate

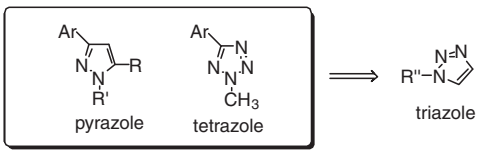

Figure 1. Structures of five-member ring MAO-Is.

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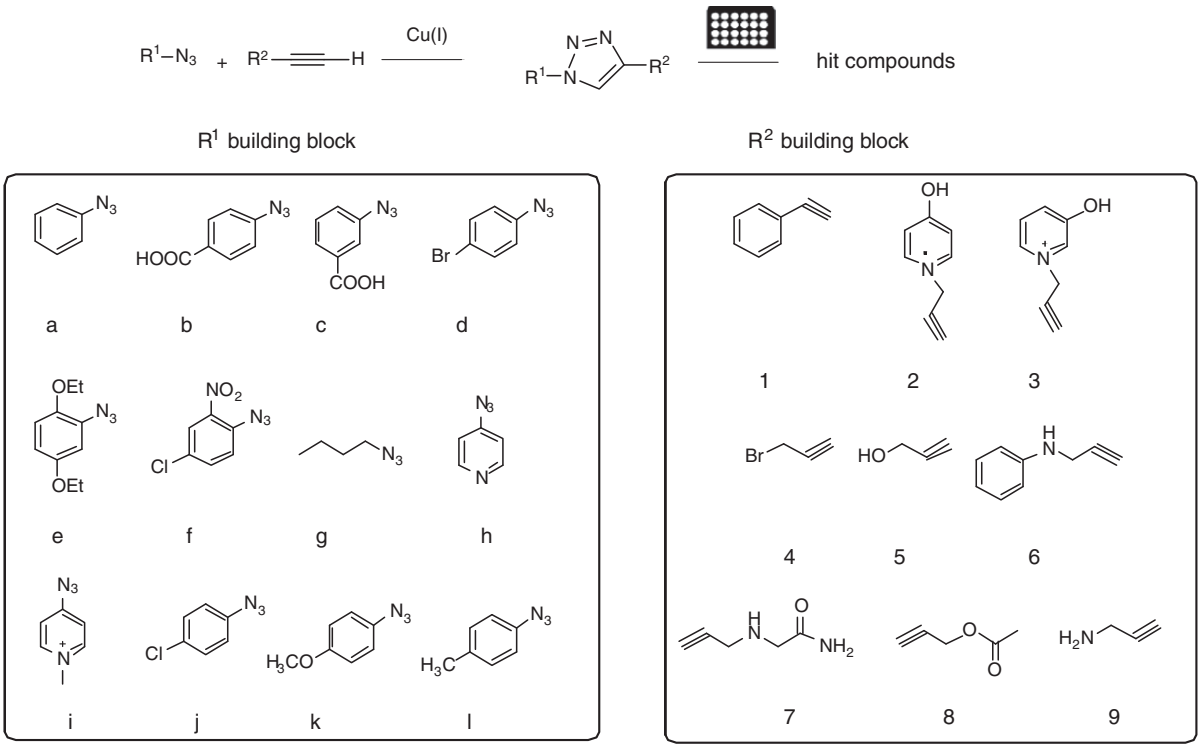

Scheme 1. Click construction of library of 1,2,3-triazoles.

screening system, the different amounts of catalysts were explored using alkyne **1** and azide **h** as a model reaction. Finally, it was found that, after incubation at room temperature for 24 h, 2% $CuSO_4$ and 20% sodium ascorbate in *t*-BuOH/H_2O (v/v 1/1) afforded full consumption of the azide core and the approximately quantitative formation of cycloaddition products. Next, the library was constructed under the following condition: a solution of compound a–**l** was dispensed into 1 mL vial containing a corresponding alkyne and catalytic amount of $CuSO_4$ and sodium ascorbate (Scheme 1). LC/MS was used for monitoring the reaction and yields ranged from 95% to 99%.[17]

The inhibitory activity of 108 compounds against MAO-A/B was determined using our reported fluorescence-based microplate assay,[18] and four hit compounds (**a9**, **b9**, **c9**, **d9**) were identified (Fig. 2). To study inhibitory efficacy, four compounds were resynthesized in larger scale and values of IC_{50} against MAO-A/B were further determined in Table 1.[19]

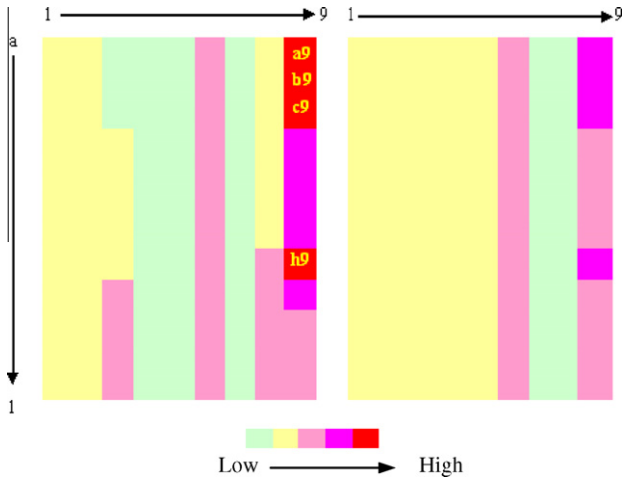

Figure 2. The fingerprint of inhibitory activity against MAO-A (left)/B (right). Strongest inhibitory activity is visualized in red according to the scale shown.

Table 1
IC_{50} for four hit compounds against MAO-A/B

Compds	MAO-A IC_{50},[a] (μM)	MAO-B IC_{50},[a] (μM)	SI[b]
a1	na	na	
a9	0.97	510.84	526.23
b9	19.62	56.88	2.89
c9	50.72	62.07	0.95
h9	0.83	115.92	139.66

[a] The results are expressed as means. Data were analyzed by one-way analysis of variance. The experiments were repeated three times.
[b] SI = MAO-B IC_{50}/MAO-A IC_{50}.

Our results reveal that compounds **h9** and **a9** are the most potent inhibitors against MAO-A, and their IC_{50} values are 0.83 μM and 0.97 μM, respectively. Meanwhile, compound **a9** shows a remarkable selectivity against MAO-A (SI = 526.63). Some further observations arose from the above screening results. First, compounds with amino group are potent inhibitors toward MAO-A, indicating that prop-2-yn-1-amine is a key building block for inhibitory activity. Second, unsubstituted aromatic rings in R^1 building block (**a9**, **h9**) have more potent inhibitory activity toward MAO-A than substituted derivatives (**b9**, **c9**). In order to shed light on the binding mode of MAO-A/B with these inhibitors, compound **a9** was computationally docked into MAO-A/B using AutoDock.[20] Crystal structures of MAO-A and B used for molecular docking were retrieved from the Protein Data Bank (PDB) as targets.[21,22]

After docking into the active site of MAO-A/B, Figure 3 illustrates the most favorable binding mode for **a9**. Firstly, the pose of **a9** shows that the phenyl ring fits into the 'aromatic cage' and sandwiched between Tyr407 and Tyr444 to establish π–π stacking interactions. Moreover, the phenyl moiety is able to establish a T-shaped π–π interaction with the FAD aromatic ring. Secondly, a hydrogen bond between the protonated amino group of compound **a9** and the car-

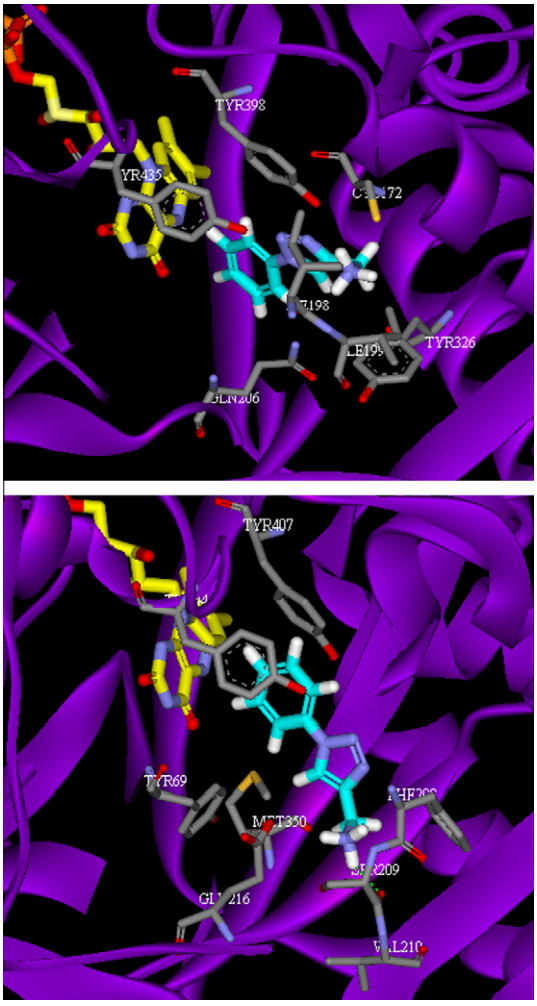

Figure 3. The interacting mode and orientations of compound **9** (in sticks at center) in the active sites of MAO-A (up), and MAO-B (down). FAD cofactor (yellow), and interacting key residues (gray) are shown in stick models. Hydrogen bonds are displayed as dashed green line.

bonyl oxygen of Ser209 side chain is observed. Thirdly, docking results demonstrate that **a9** is embedded in a large hydrophobic pocket formed by Tyr 69, Phe208, Val210, Gly216 and Met350. Unexpectedly, in the active site of MAO-B, the phenyl ring is positioned underneath the enzymatic 'aromatic cage' formed by Tyr398, Tyr435 and FAD, thus **a9** fails to form any π–π stacking interaction with MAO-B. In addition, docking study also displays that no hydrogen bond is observed between the enzyme and the inhibitor.

In conclusion, an efficient strategy for the fast construction 108 compounds library was developed using click chemistry. The fingerprint of inhibitory activity toward MAO-A/B against this library was obtained, and four hit compounds, especially **a9** exhibited excellent inhibitory activity and selectivity toward MAO-A. Docking study was carried out to demonstrate the binding mode between **a9** and MAO-A/B, and the result reveals that compound **a9** can establish π–π stacking interactions with Tyr407, Tyr444 and FAD in MAO-A rather than in MAO-B. We expect this strategy will contribute greatly to develop more selective and potent inhibitors against MAO-A as well as MAO-B.

Acknowledgments

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14. *Experimental procedure for alkyne* **2** *and* **3**: the pyridine (1 mmol) was dissolved in dry dichloromethane and 3-bromopropyne (1.5 mmol) was added. The mixture was stirred at room temperature over night to afford the desired products as the resultant precipitate.
4-Hydroxy-1-(prop-2-ynyl) pyridinium (**2**): yield 75%. ^{1}H NMR (D_2O): δ 7.41–7.39 (d, 2H, *J* = 5 Hz, CH), 6.35–6.34 (d, 2H, *J* = 5 Hz, CH), 4.56 (s, 2H, CH_2), 2.59–2.58 (d, 1H, *J* = 5 Hz, C≡CH). MS (ESI) *m/z* 134.1 (M^+).
3-Hydroxy-1-(prop-2-ynyl) pyridinium (**3**): yield 67%. ^{1}H NMR (D_2O): δ 8.32–8.29 (m, 2H, CH), 7.80–7.78 (m, 1H, CH),7.74–7.71 (m, 1H, CH),5.27 (s, 2H, CH_2), 3.11–3.10 (d, 1H, *J* = 5 Hz, C≡CH). MS (ESI) *m/z* 134.1 (M^+).
15. *Typical experimental procedure for aryl azide*: 4-bromide aniline (0.15 mol) was dissolved in (40 ml) 50% H_2SO_4 at 0–5 °C, followed by addition of sodium nitrite (0.20 mol). After 1 h, sodium azide (0.3 mol) dissolved in 15 ml of water was added drop wise, and the reaction is allowed to continue over night. The resultant precipitate was extracted with chloroform and washed successively with water. The organic layer was dried over anhydrous sodium sulfate, and the solvent stripped out in rotary evaporator to get crude product. The residue was chromatographed on silica gel using 10% EtOAc in hexane to give 1-Azido-4-bromobenzene (**d**): yield 84%. ^{1}H NMR ($CDCl_3$): δ 7.48–7.46 (d, 2H, *J* = 5 Hz, CH), 6.92–6.91 (d, 2H, *J* = 5 Hz, CH),. MS (EI) *m/z* 197.1 (M^+, 100%), 199.1 (M^++2, 96%). IR (KBr, cm^{-1}): 2137 (–N_3).
16. *Preparation of compound* **h** *and* **i**: To a solution of 4-bromo-pyridine hydrochloride (20 mmol) in ethanol/water = 1/1 (40 ml) was added NaOH (0.4 g) and NaN_3 (3 g). The reaction mixture was refluxed at 110 °C for 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was distilled, and the mixture was extracted with dichloromethane. The organic layer was separated, washed with brine (20 ml) and saturation $NaHCO_3$ (20 ml), anhydrous $MgSO_4$ dried. The solvent was evaporated to afford product **h**. Compound **h** was dissolved dry dichloromethane and MeI (2.5 ml) was added. The mixture was stirred at room temperature over night. The resultant precipitate was filtered to afford product **i**.
4-Azidopyridine (**h**): yield 86%. ^{1}H NMR ($CDCl_3$): δ 8.50–8.48 (m, 2H, CH), 7.65–7.63 (m, 2H, CH). MS (ESI) *m/z* 121.2 $(M+H)^+$. IR (KBr, cm^{-1}): 2121 (–N_3).
4-Azido-1-methylpyridinium (**i**): yield 93%. ^{1}H NMR(D_2O): δ 8.49–8.47 (m, 2H, CH), 7.50–7.48 (m, 2H, CH), 4.14 (s, 3H, CH_3). MS(ESI) *m/z* 135.2 (M). IR (KBr, cm^{-1}): 2119 (–N_3).
17. *Microplate-based screening of MAO inhibitors*: 20 μL of azide (10 mM) and a corresponding alkyne (1.0 equiv) were dispensed. Ten microliter of $CuSO_4$ (400 μM), and 10 μL of sodium ascorbate (4 mM) were added, and *t*-BuOH/H_2O (v/v 1/1) was added to keep the mixture at 200 μL. The reaction was monitored by LC–MS and was completed after 24 h.
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19. *General procedure for the Cu^+-catalyzed [3+2] cycloaddition of azides and alkynes*: Azide (1.6 mmol, 1.2 equiv) and then alkyne (1.3 mmol, 1.0 equiv) were suspended in a 1:1 mixture of water and *t*-BuOH (1.5 mL each) in a 10-mL one-neck round bottle equipped with a small magnetic stirring bar. To this was added copper sulfate solution (1 M, 20 μL) and sodium ascorbate solution (1 M, 200 μL). The mixture was stirred for 24 h at room temperature, after which time TLC analysis indicated complete consumption of starting materials. The solvent was distilled, water (20 mL) was added, and the mixture was extracted with dichloromethane. The organic layer was separated, washed with brine (20 mL) and saturation $NaHCO_3$ (20 ml), anhydrous $MgSO_4$ dried. The solvent was evaporated to afford crude product, which was further purified by alumina column chromatography to offer product as a yellow solid.
(1-(Pyridin-4-yl)-1H-1,2,3-triazol-4-yl) methanamine (**h9**): 91%. ^{1}H NMR(D_2O): δ 8.55–8.54 (m, 2H, CH), 8.35 (s, 1H, –CH═C), 7.71–7.70 (m, 2H, CH), 3.85 (s, 2H, CH_2). MS (ESI) *m/z* 176.1 $(M+H)^+$.
(1-Phenyl-1H-1,2,3-triazol-4-yl)methanamine (**a9**): 95%. ^{1}H NMR(D_2O): δ 8.02 (s, 1H, –CH═C), 7.44–7.43 (m, 2H, CH), 7.37–7.31 (m, 3H, CH), 3.75 (s, 2H, CH_2). MS (ESI) *m/z* 175.1 $(M+H)^+$.
4-(4-(Aminomethyl)-1H-1,2,3-triazol-1-yl)benzoic acid (**b9**): 93%. ^{1}H

NMR(DMSO): δ 8.95 (s, 1H, –CH═C), 8.18–8.16 (m, 2H, CH), 8.06–8.04 (m, 2H, CH),4.25 (s, 2H, CH_2). MS (ESI) *m/z* 219.1 $(M+H)^+$.
3-(4-Aminomethyl-[1,2,3]triazol-1-yl)-benzoic acid (**c9**): 93%. ^{1}H NMR(DMSO): δ 8.87 (s, 1H, –CH═C), 8.33 (s, 1H, CH), 8.02–8.02 (m, 2H, CH),7.69 (s, 1H, CH), 4.21 (s, 2H, CH_2). MS (ESI *m/z* 219.1 $(M+H)^+$.

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