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Design and characterization of bivalent compounds as potential neuroprotectants for Alzheimer's disease: Impact of the spacer on biological activity

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ABSTRACT

In our continuing efforts to develop bivalent compounds as potential neuroprotectants for Alzheimer's disease, a series of bivalent compounds that contain cholesterylamine and an extended spacer were synthesized and biologically characterized. Our results demonstrated that incorporation of a piperazine ring into the spacer composition significantly improved the protective potency in MC65 cell models. Our results also suggested that the optimal spacer length for such bivalent compounds ranges from 17 to 21 atoms, and further spacer extension beyond 21 atoms results no further optimization. Notably, incorporation of a piperazine ring into the spacer diminished the biometal chelating capacity for these bivalent compounds, thus suggesting structural flexibility of these compounds in interactions with metals. Collectively, the results provided valuable guidance to develop new bivalent compounds as neuroprotectants for Alzheimer's disease.

Recent research have revealed the critical roles of multiple pathogenic factors in the development of Alzheimer's disease (AD), a devastating neurodegenerative disorder without cure.[1] This includes protein aggregation including beta-amyloid (Aβ) and tau, oxidative stress, neuroinflammation, and mitochondrial dysfunction, among others.[2–6] Although significant advances have been made to understand the basic mechanisms leading to AD, the etiology of this disease still remains elusive. Consequently, the complex nature of this disease made drug discovery efforts still an unmet task. Recently, small molecules with potential multifunctional properties have attracted attention to provide effective disease-modifying agents as AD therapeutics.[7–9]

Our group has developed a bivalent strategy by linking a multifunctional "warhead" curcumin with a steroid type membrane-anchoring moiety via a spacer to explore their potential as AD therapeutics.[10–15] This strategy was constructed based on the fact that curcumin is an important phytochemical with antioxidant, anti-inflammatory and anti-Aβ activities;[16,17] and sterol analogs such as cholesterol, dihydrocholesterol and cholesterylamine have demonstrated utility as membrane anchors.[13,15,18–20] Our results demonstrated the superior protective activities of the bivalent compounds compared to the "warhead" curcumin or anchoring steroid alone or the combination of these two.[10–15] Furthermore, our studies suggested the important roles of the spacer that links the "warhead" and the anchoring moiety to the observed biological activity, consistent with the reported results.[19] Our studies also identified an optimal spacer length ranging from 17 to 21 atoms to provide potent bivalent compounds.[11,13,15] Characterization of bivalent compounds using diosgenin as the anchoring moiety suggested that spacer length beyond 26 atoms will lead to decreased protective potency.[11] Given the structural flexibility of the bivalent structure due to the spacer linkage, it is not clear whether the length of the spacer can be further extended and how the change of spacer composition will impact the biological activity. Herein, we report the synthesis and biological characterization of a series of bivalent compounds containing cholesterylamine as the anchoring moiety and an extended spacer (from 21 atoms to 39 atoms with 2 atom increment each time) as model compounds to understand the impact of spacer length extension on their biological activities.

In the design of this series of bivalent compounds, we incorporated a piperazine moiety into the spacer (Fig. 1) with the following reasons: 1) to increase the structural constrain of the spacer, consequently reduce the overall structural flexibility of bivalent compounds; 2) to improve the overall solubility of the designed compounds by incorporating two basic nitrogen atoms from the piperazine ring. As shown in Fig. 1, the spacer length varies by 2-atom increment each time starting with 21 atoms and the max-

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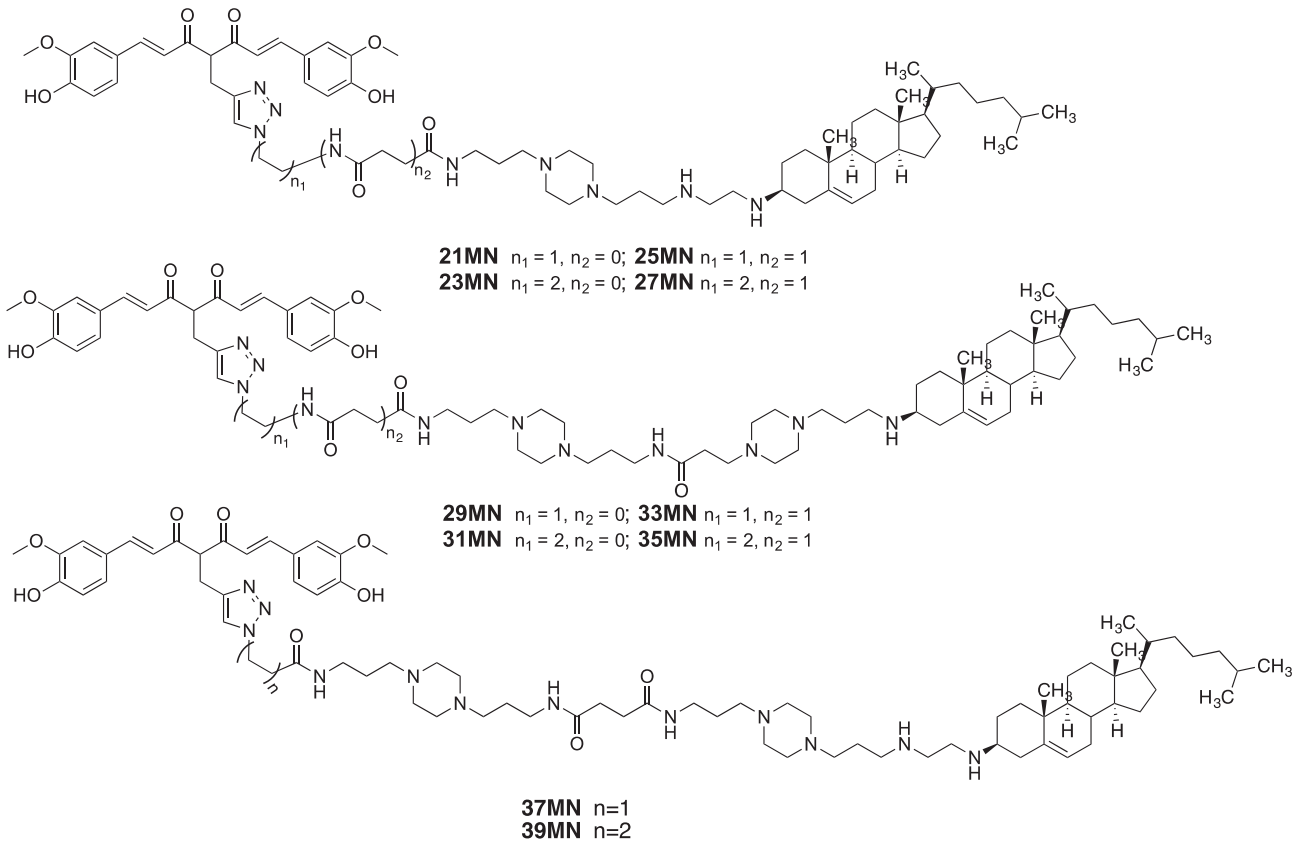

Fig. 1. Chemical structures of the designed bivalent compounds 21MN–39MN.

imal length is 39 atoms. For the compound coding, the Arabic numeral represents the number of atoms in the spacer, the "M" represents the connecting position of spacer on the curcumin moiety is on the "middle" position between the two carbonyl groups, and the "N" represents the connection of the spacer to the anchoring moiety is through a nitrogen.

The chemical syntheses of 21MN-27MN were achieved by following conditions outlined in Scheme 1. Briefly, reaction of cholesterylamine **1** and N-2-bromo-ethyl-phthalimide followed by Boc protection gave intermediate **2**, which was refluxed with NH2NH2 in ethanol followed by reaction with 2-nitrobenzenesulfonyl (Nosyl) chloride to give N-protected intermediate **3**. Reaction of **3** with N-(4-(3-bromopropyl)-1-yl) -propyl-phthalimide, which was prepared from 3-(piperazin-1-yl) propan-1-ol, followed by the removal of the Nosyl group in the presence of thiophenol gave intermediate **4**. Boc protection of **4** followed by removal of the phthalimide protecting group yielded the common precursor **5**. Coupling reactions of **5** with various azido-carboxylic acids afforded azido-intermediates **6**, **7**, **8**, and **9**. The click reaction of alkyne **10**, obtained as previously reported,[15] with **6**, **7**, **8**, or **9** followed by removal of the Boc protecting group in the presence of trifluoroacetic acid (TFA), gave bivalent compounds **21MN**, **23MN**, **25MN**, or **27MN**, respectively.

As shown in Scheme 2, reaction of **1** with **11**, prepared from 3-(piperazin-1-yl)propan-1-ol with methyl 3-bromopropanoate, followed by Boc protection afforded intermediate **12**. Hydrolysis of **12** with LiOH in MeOH/H_2O followed by coupling reaction with N-(4-(3-aminopropyl)piperazin-1-yl)propyl- phthalimide yielded **13**, which was refluxed with NH_2NH_2 in ethanol to afford intermediate **14**. Reaction of **14** with various azido-carboxylic acids gave azido-intermediates **15–18**. Click reactions of alkyne **10** with **15–18** followed by removal of the Boc protecting group gave bivalent compounds **29MN**, **31MN**, **33MN**, and **35MN**.

The syntheses of **37MN** and **39MN** were outlined in Scheme 3. Briefly, coupling reaction of **19**, which was prepared by reaction of N-(4-(3-bromopropyl)-1-yl)-propyl-phthalimide with succinic anhydride, with **5** gave intermediate **20**. Refluxing of **20** with NH_2-NH_2 in ethanol followed by coupling reaction with 3-azido-propanoic acid or 5-azido-pentanoic acid yielded **21** and **22**, respectively. Lastly, the click reaction of **21** or **22** with alkyne **10** followed by removal of the Boc protecting group afforded **37MN** and **39MN**, respectively.

After chemical synthesis, this series of bivalent compounds were tested to evaluate their protective potency to rescue MC65 cells. MC65 is a neuronal cell line that conditionally expresses C99, the C-terminus fragment of amyloid precursor protein (APP).[20,21] Upon tetracycline (TC) withdrawal, MC65 cells produce intracellular Aβ aggregates and oxidative stress, two of the suggested pathogenic factors in AD development, ultimately leading to cell death. Studies from our group and others have demonstrated this cellular assay is a suitable primary screening model to identify small molecule compounds with potential neuroprotective activities.[13,15,22–25] As shown in Table 1, compound **21MN** exhibited protection in the MC65 cell assay with an EC_{50} of 23.85 ± 2.26 nM, ~10-fold increase compared to another bivalent compound we previously reported thatcontains a 21-atom spacer but different spacer composition.[13] This is in agreement with our recent studies to show that the spacer composition is a key factor in optimizing protective potency.[28] This also suggests that incorporation of a piperazine moiety into the spacer represents a means to improve potency for future structural modifications. Overall, the increase of spacer length from 21 to 37 atoms did not significantly change the protective potency (Table 1). As evidenced by **39MN**, when the spacer reached to a 39-atom length, the protective potency dropped to 93.98 ± 3.68 nM, 4-fold decrease compared to that of **21MN**. Combining with the results fromour previous stud-