Synthesis of Phidianidine Analogues Containing a 1,2,3-triazole

David Laws III, Dillon King and Bryan H. Wakefield

Coastal Carolina University, Conway, SC

Phidianidines are a class of compound that has been extracted from the sea mollusk *Phidianis militaris*. These compounds have been shown to exhibit a variety of useful properties such as antihistamine effects, anti-cancer activity, agonism of the μ-opioid receptor and neuroprotection. The biological activities are thought to be caused by the 1,2,4- oxadiazole ring found within the molecule. The goal of this project is to synthesize analogues of phidianidine that contain a 1,2,3-triazole instead of the 1,2,4 oxadiazole ring using a method that will allow for other regions of the molecule to be changed. This will help to elucidate the role of the 1,2,4-oxadiazole ring in biological activity as well as probe if the 1,2,3-triazole analogue can provide significant improvement to any activities.

Introduction

Marine organisms have been proven a rich source for bioactive molecules over the last 50 years. Many of the marine natural products discovered belong to a class of compounds called alkaloids.[1-3] Alkaloids are produced widely by both terrestrial and marine organisms and evolved as a defense mechanism. For this reason alkaloids are often cytotoxic. A subset of alkaloids, indole alkaloids, are important compounds in the field of drug discovery that commonly possess neurological activity.[4,5] One such group of indole alkaloids are the phidianidines, which were isolated by Gavagnin in 2011 from the sea mollusk *Phidianis militaris*. Phidianidines A and B (Figure 1), which differ based on the presence of a bromide, possess a unique 1,2,4-oxadizole ring and biological properties.[6]

The phidianidines have a range of interesting bioactivity such as anticancer[6], HIV and neurological activity.[7,8] Phidianidine A binds to the chemokine receptor CXCR4. CXCR4 is an important target in the field of pharmacology due to its involvement in HIV progression, tumor development and metastasis, and rheumatoid arthritis. Phidianidine A was shown to bind to CXCR4 moderately with hopes of improving its activity.[7] Additionally; the phidianidines have interesting applications in neurological disorders. They function as a selective inhibitor of the dopamine transporter and a selective ligand of the μ-opioid receptor.[8] Selectivity among opioid receptors is important because the δ- and κ-opioid receptors are linked to addiction, while the μ-opioid receptor is responsible for the analgesic effects.[9,10] Other projects investigated the neuroprotective activities of phidianidine derivatives, and found that 4-substituted phidianidine derivatives offer significant protection against hydrogen peroxide and oxygen-glucose deprivation in vitro which can impact the onset and progression of neurodegenerative diseases like Alzhiemer's.[11] Given these several important biological activities, synthesis of phidianidine analogues are essential to understanding and improving its functions.

Phidianidines are composed of three distinct regions, an indole, a 1,2,4-oxadiazole ring, and an aminoalkylguanidine chain. The bioactivity is mainly thought to be a result of the 1,2,4-oxadiazole ring, and phidianidine is the first isolated natural product to contain this structure.[6] Previous synthesis of the phidianidines used a hydroxyguanidine linker with a protected nitrogen and ethyl indole-3-acetate to form the 1,2,4-oxadiazole ring.[12,13] This has limited the ability of groups to explore the affect of structural changes to the 1,2,4-oxadiazole and indole on the biological activity. We hope to develop an approach to phidianidine analogues that addresses this limitation.

The overall goal of this project is to synthesize a 1,2,3-triazole analogue of the phidianidines (Figure 2) via cyclization of an azido-group with a 3-propargyl indole.[14] This approach will allow for the impact of changes to the 1,2,4-oxadiazole ring and indole on the molecule's biological activity to be determined. The identification of cyclization conditions to be used for the synthesis of the 1,2,3-triazole analogues is reported herein.

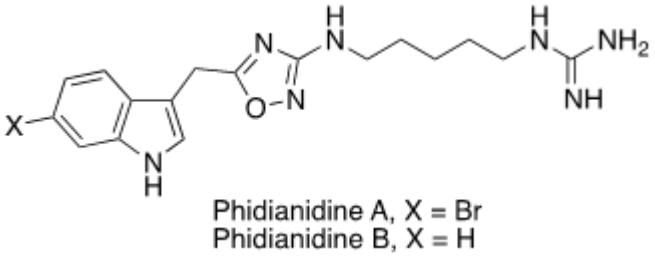


Figure 1. Structure of phidianidine A and B

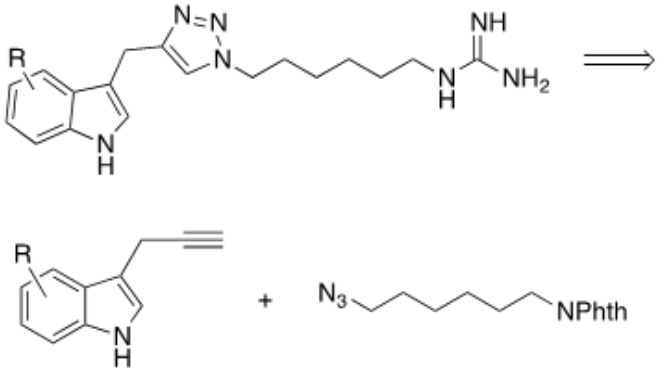


Figure 2. The key step to the 1,2,3-triazole analogues of the phidianidines

Scheme 1. Synthesis of 3-propargyl indoles

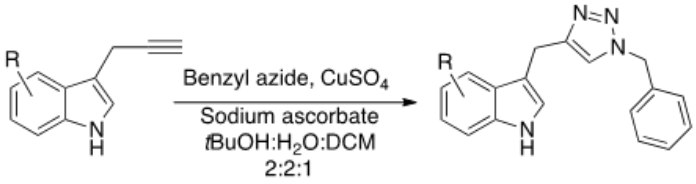
Table 1. Results of 3-propargyl indole syntheses

Entry	R	Yield
1	H	84%
2	2-methyl	43%
3	4-methyl	96%
4	6-bromo	87%
5	7-methyl	86%

Chemistry

In the reaction scheme, indole was reacted with propargyl bromide to produce 3-propargyl indole (Scheme 1). This reaction was successfully realized with a variety of indoles (Table 1). To determine the optimum conditions for the synthesis of the 1,2,3-triazole heterocycle,

commercially available benzyl azide was used as a model (Scheme 2). Treatment of the alkyne and azide under standard cycloaddition conditions gave poor results[15] (Table 2, entry 1). No product was isolated though this could be due to difficulties in purification. In order to improve the yield, the equivalents of the reactants were doubled and the temperature increased to 60˚C. This provided moderate increases to yield in some cases, however the yield was still around 30% (Table 2, entries 2-4). The temperature and equivalents were increased again (80˚C, 4 equiv.) however the yield decreased to 8% (Table 2, entry 5). It was hypothesized that the low yields were caused by oxidation of the Cu (I) catalyst by O_2 gas within the solvent. The solvent was degassed with bubbling N_2; this led to the heterocycle being successfully synthesized in 59% yield (Table 2, entry 6). The cyclization was also tested with various indoles with less success (Table 2, entries 7-10). Using benzyl azide for the reaction allowed for reaction conditions to be developed, but provided a truncated analogue of the natural product missing the guanidine functionality. Work is currently underway to identify more general cyclization conditions and to construct the azide needed to synthesize the full analogue.



Scheme 2. Synthesis of the 1,2,3-triazole ring

Methods

General Procedure for the synthesis of 3-propargyl indoles
A solution of 3.0 M MeMgI in ether (1.1 equiv) was added to benzene and heated to 40 ˚C. To this solution was slowly added the appropriate indole in 2 mL benzene. After 10 min, propargyl bromide (0.7 equiv) was added. The reaction stirred overnight at reflux then quenched with NH_4Cl, extracted with EtOAc (2x). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*.[16]

3-propargylindole
After completing the general procedure with 20 mL of benzene and 20 mmol of indole, the residue was purified via flash chromatography on SiO_2 (1:9, EtOAc:Hexanes) to yield 1.72 g (84%) as a red-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (bs, 1 H), 7.66 (d, 1 H, *J* = 8.0 Hz), 7.38 (d, 1 H, *J* = 8.0 Hz), 7.25-7.19 (m, 2 H), 7.16 (t, 1H, *J* = 8.0 Hz), 3.72 (s, 2 H), 2.17-2.14 (m, 1 H).

3-propargyl-2-methylindole
After completing the general procedure with 5 mL of benzene and 5 mmol of 2-methylindole, the residue was purified via flash chromatography on SiO_2 (1:9, EtOAc:Hexanes) to yield 0.24 g (43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (bs, 1 H), 7.68 (d, 1 H, *J* = 8.0 Hz), 7.24-7.14 (m, 3 H), 3.67 (s, 2 H), 2.47 (s, 3 H), 2.09-2.08 (m, 1 H).

6-bromo-3-propargylindole
After completing the general procedure with 5 mL of benzene and 5 mmol of 6-bromoindole, the residue was purified via flash chromatography on SiO_2 (1:9, EtOAc:Hexanes) to yield 0.66 g (87%) as a dark brown solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.02 (bs, 1 H), 7.53-7.49 (m, 2 H), 7.25 (d, 1 H, J = 8.4 Hz), 7.16 (s, 2 H), 2.17-2.14 (m, 1 H).

3-propargyl-7-methylindole
After completing the general procedure with 5 mL of benzene and 5 mmol of 7-methylindole, the residue was purified via flash chromatography on SiO_2 (1:9, EtOAc:Hexanes) to yield 0.47 g (86%) as a brown solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.01 (bs, 1 H), 7.59 (d, 1 H, *J* = 7.6 Hz), 7.20-7.08 (m, 3 H), 3.8 (s, 2 H), 2.58 (s, 3 H), 2.24-2.23 (m, 1 H) .

Synthesis of 1,2,3-triazole with benzyl azide
To a mixture of 3-propargyl indole (50 mg, 0.30 mmol) in 4 mL of H_2O: tBuOH: DCM (2:2:1) was added benzyl azide (0.04 mL, 0.33 mmol) and $CuSO_4$ (7 mg, 0.04 mmol). The reaction was then degassed via bubbling N_2 for 30 min. The reaction was then treated with sodium ascorbate (26 mg, 0.13 mmol) and stirred overnight. The organic was extracted with DCM (3x), washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*.[15] The residue was purified via chromatography on SiO_2 (1:9 to 4:6 EtOAc:Hexanes) to yield 25 mg (59%) of an orange-brown oil. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.05 (bs, 1 H), 7.51 (d, 1 H, 7.6 Hz), 7.39-7.29 (m, 4 H), 7.24-7.16 (m, 3 H), 7.15-7.05 (m, 3 H), 5.45 (s, 2 H), 4.23 (s, 2 H).

Results and Conclusion

The initial steps toward the synthesis of 1,2,3-triazole analogues of phidianidine have been realized. Addition of propargyl to the 3-position of indole has been achieved with variety of indoles, with yields between 80-90%. This opens a route for a variety of 1,2,3-triazole analogues to be synthesized from various indoles. Synthesis of the 1,2,3-triazole heterocycle was realized with 59% yield though more work is needed to expand the substrate scope. In addition to this, the synthesis of an azide that will yield guanidine side chain is ongoing.

Table 2. Optimization of the cycloaddition reaction

Entry	R	Benzyl Azide equiv.	Sodium Ascorbate equiv.	$CuSO_4$ equiv.	Temp	Yield
1	H	1.1	0.45	0.15	RT	----------
2	H	2.2	0.9	0.3	60˚C	31%
3	H	2.2	0.9	0.3	60˚C	35%
4	H	2.2	0.9	0.3	60˚C	8%
5	H	4.4	1.8	0.6	80˚C	28%
6	H	1.1	0.45	0.15	RT	59%
7	H	2.2	0.9	0.3	60˚C	8%
8	6-bromo	1.1	0.45	0.15	RT	17%
9	7-methyl	1.1	0.45	0.15	RT	25%
10	2-methyl	1.1	0.45	0.15	RT	14%

Acknowledgements

The authors would like to thank South Carolina INBRE and the National Institute of Health for supporting this research (NIH 5P20GM103499).

Notes and References

**Corresponding author email: bwakefiel@coastal.edu*

1. Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. 2017. Marine natural products. Nat. Prod. Rep. 34:235-294.
2. Andersen RJ. 2017. Sponging off nature for new drug leads. Biochem. Pharmocol. http://dx.doi.org/10.1016/j.bcp.2017.04.012.
3. Sugumaran M, Robinson WE. 2010. Bioactive Dehydrotyrosyl and Dehydrodopyl Compounds of Marine Origin. Mar. Drugs. 8:2906-2935.
4. Kochanowska-Karamyan AJ, Hamann MT. 2010. Marine Indole Alkaloids: Potential New Drug Leads for the Control of Depression and Anxiety. Chem. Rev. 110:4489-4497.
5. Wahba AE, Hamann MT. 2010. New One-Pot Methodologies for the Modification or Synthesis of Alkaloid Scaffolds. Mar. Drugs. 8:2395-2416.
6. Carbone M, Li Y, Irace C, Mollo E, Castelluccio F, Pascale AD, Cimino G, Santamaria R, Guo YW, Gavagnin M. 2011. Structure and Cytotoxicity of Phidianidines A and B: First Finding of 1,2,4-Oxadiazole System in a Marine Natural Product. Org. Lett. 13:2516-2519.
7. Vitale RM, Gatti M, Carbone M, Barbieri F, Felicita V, Gavagnin M, Florio T, Amodeo P. 2013. Minimalist Hybrid Ligand/Receptor-Based Pharmacophore Model for CXCR4 Applied to a Small-Library of Marine Natural Products Led to the Identification of Phidianidine A as a New CXCR4 Ligand Exhibiting Antagonist Activity. ACS Chem. Biol. 8:2762-2770.
8. Brogan JT, Stoops SL, Lindsley CW. 2012. Total Synthesis and Biological Evaluation of Phidianidines A and B Uncovers Unique Pharmacological Profiles at CNS Targets. ACS Chem. Neurosci. 3:658-664.
9. Klenowski P, Morgan M, Bartlett SE. 2015. The role of δ-opioid receptors in learning and memory underlying the development of addiction. Br. J. Pharmacol. 172:297-310.
10. Simonson B, Morani AS, Ewald AM, Walker L, Kumar N, Simpson D, Miller JH, Prisinzano TE, Kivell BM. 2015. Pharmacology and anti-addiction effects of the novel κ opioid receptor agonist Mesyl Sal B, a potent and long-acting analogue of salvinorin A. Br. J. Pharmacol. 172:515-531.
11. Jiang CS, Fu Y, Zhang L, Gong JX, Wang ZZ, Xiao W, Zhan HY, Guo YW. 2015. Synthesis and biological evaluation of novel marine-derived indole-based 1,2,4-oxadiazoles derivatives as multifunctional neuroprotective agents. Bioorg. Med. Chem. Lett. 25:216-220
12. Lin HY, Snider BB. 2012. Synthesis of Phidianidines A and B. J. Org. Chem. 77:4832-4836.
13. Manzo E, Pagano D, Carbone M, Ciavatta ML, Gavagnin M. 2012. Synthesis of Phidianidine B, a highly cytotoxic 1,2,4-oxadiazole marine metabolite. ARKIVOC 9:220-228.
14. Totobenazara J, Burke AJ. 2015. New click-chemistry methods for 1,2,3-triazoles synthesis: recent advances and applications. Tet. Lett. 56:2853-2859.
15. Minvielle MJ, Bunders CA, Melander C. 2013. Indole–triazole conjugates are selective inhibitors and inducers of bacterial biofilms. Med. Chem. Commun. 4:916-919.
16. Youn SW, Pastine SJ, Sames D. 2004. Ru(III)-Catalyzed Cyclization of Arene -Alkene Substrates via Intramolecular Electrophilic Hydroarylation. Org. Lett. 6:581-584.