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An efficient one-pot synthesis of tethered cyclohexadiene enaminonitriles from methyl-ketones: An effective route to quinazolines

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Abstract—The quinazolines represent a useful natural product scaffold with demonstrated activities against disorders such as high blood pressure and benign prostatic hyperplasia. Here we report on the synthesis and biological activity of a series of quinazolines that were prepared by a one-pot synthesis of substituted cyclohexadiene enaminonitriles from methyl-ketones. The approach, which employs NaH, complements published procedures where LDA is utilized. While the NaH catalyzed reaction generates the cyclohexadiene enaminonitriles in high yields with heterocyclic substrates, the reaction fails to promote product formation of aliphatic alkyl substrates. On the contrary, the LDA mediated synthesis favors the long chain alkyl substituents while reactions involving the aromatic substrates result in low yields. The final conversion to the quinazolines is also a modification on literature protocols.
In cellular assays, the quinazolines showed the most promising activity against Jurkat with CC_{50} values in the low micromolar range. Weak activity was observed against microbial strains (*Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae*). The substituted enaminonitrile intermediates also exhibited weak anti-microbial activity and cytotoxicity against human T-cell leukemia.

Heterocyclic systems have achieved prominence as pharmaceuticals, fungicides, and sovatochromatic dyes.[1–4] The quinazolines (Fig. 1), for example, have been implemented to treat high blood pressure (Doxazosin **1** and Prazosin **2**) and benign prostatic hyperplasia (Alfuzosin **3**).[5–12] Likewise, enaminonitriles, such as **4** and its analogues **5**–**7** (Fig. 2), have shown biological activity (**4**, 18 μM; **5**, 35 μM; **6**, 19 μM; **7**, 16 μM) against scrapie-infected mouse neuroblastoma cells (ScN2a) inhibiting PrP^{Sc},[13] an isoform of the cell-surface glycosylphosphatidylinositol (GPI)-anchored protein PrP^{c}.[14]

In this investigation, we report on the synthesis of quinazolines generated from the a cyclohexadiene enaminonitrile scaffold (Fig. 3) that was in turn prepared by a one-pot strategy involving treatment of methyl-ketones with excess NaH. We compare this synthetic methodology with that reported previously, where self-dimerization of α,β-unsaturated nitriles can be invoked by treatment with lithium diisopropylamide (LDA)[15] or other strong bases to give the enaminonitriles (Fig. 4) in moderate yields.[16,17] We additionally report on the biological activity of these compounds including their cytotoxic effects against bacteria (*E. coli* and *B. subtilis*), yeast, and human T-cell leukemia (Jurkat). Interestingly, members of a functionalized cyclohexadienal library have shown their ability to stimulate neurite outgrowth, while others have demonstrated anti-cancer activity.[18,19]

Enaminonitriles can be generated in a one-pot format by implementation of a Horner–Wadsworth–Emmons reaction (Fig. 5). When methyl-ketones are treated with an ylide solution generated by treatment of diethyl (cyanomethyl)-phosphonate with one equivalent of sodium hydride, conversion to the α,β-unsaturated nitrile is observed.[21] However, in the presence of excess sodium hydride, the reaction proceeds to give the enaminonitrile.

To optimize the synthesis of our enaminonitriles, we utilized **8** as a model substrate, where it was found that six

Keywords: Enaminonitrile; Quinozoline; Horner–Wadsworth–Emmons; Cell-based assays.
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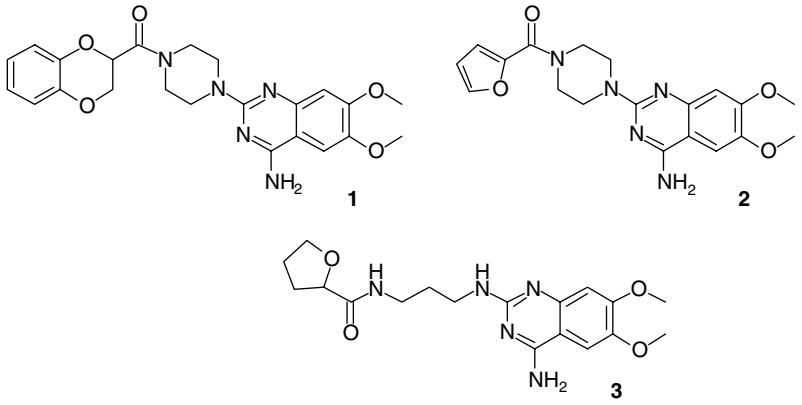


Figure 1. Quinazolines used as therapeutic agents.

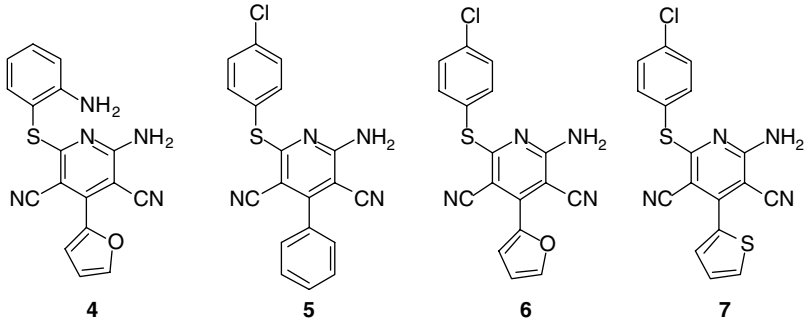


Figure 2. Enaminonitriles active against mice ScN2a cells.

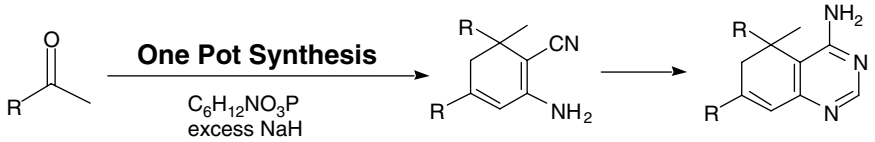


Figure 3. Quinazalines derived from a cyclohexadiene enaminonitrile scaffold.

excess equivalents of NaH were required to facilitate the deprotonation of the nitrile (β-methyl group, Fig. 6) to give an 89% yield of the enaminonitrile after 4 days. When fewer equivalents were used, 0.5, 1.0, 1.25, and 2.0 equiv, no product was obtained while 3.0 and 4.0 equiv gave yields of 7% and 43%, respectively. At higher concentrations, 8.0 and 10.0 equiv of NaH, 41% and 10% of product were isolated. Presumably, the high concentration of base drives the majority of the nitrile to the deprotonated or β-methylenic form, diminishing product formation (Fig. 6). Time course analysis also revealed that 4 days was optimal as yields diminished before and after that point.

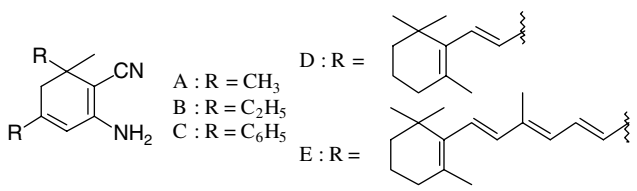


Figure 4. Known cyclohexadiene enaminonitriles.[20]

With these optimized conditions in hand, various substrates were tested to investigate the scope of the reaction as shown in Table 1. Product yields ranged from 79% to 96% for substrates containing small aromatics and bulky groups while aliphatic alkyl substrates gave no product. Thiophene **8** gave a yield of 89%, while the bromo-substituted thiophene **9** was generated in almost quantitative yield, 96%. With phenyl and its substituted derivatives, bromo-phenyl **11** exhibited the lowest yield, 79% as compared to 88% for both the phenyl **9** and fluoro-phenyl **10** substituents. Two of the higher yielding substrates selected were consisting of bulky substrates, biphenyl **12** and naphthalene **13**, giving product yields of 93% and 90%, respectively.

The primary limitation of this one-pot strategy is that it does not facilitate the self-dimerization of aliphatic alkyl substrates. When either β-ionone **15** or 2-octone **16** was employed, we found that no dimer product was seen in the crude ^{1}H NMR with the only reaction product being the nitrile intermediate itself. This could be due to reduced stabilization of the resulting β-methyl anion, requiring a stronger base to deprotonate the methyl group.

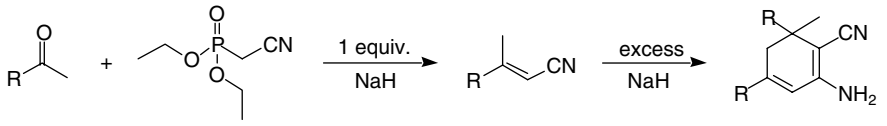


Figure 5. Horner–Wadsworth–Emmons reaction; 1.1 equiv $C_6H_{12}NO_3P$, 1.1 or 7.1 equiv NaH.

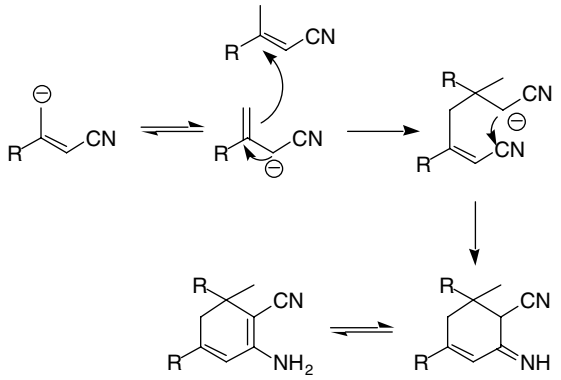


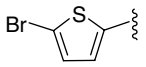
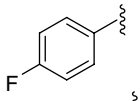
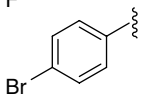
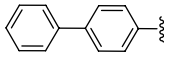
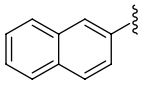
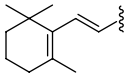
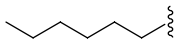
Figure 6. Proposed mechanism of reaction.

To evaluate the effectiveness of our NaH catalyzed reaction, we analyzed the formation of enaminonitriles from α,β-unsaturated nitriles using LDA in tetrahydrofuran (THF) (Fig. 7).[15] In this case, the nitrile substrates are generated from their corresponding methyl-ketones, isolated, and subsequently reacted with LDA, thus producing the enaminonitriles in two steps. We saw an inverse correlation with LDA as compared to the NaH reaction (Table 1). The NaH reaction readily dimerizes aromatics and bulky substrates in high yields, while LDA gives minimal yields, presumably reflecting steric effects, the lack of full dissociation of the catalyst prior to self-condensation. Conversely, reactions catalyzed with LDA seemed to favor long chain alkyl substituents. Previous reports of LDA dimerization with alkylidene malononitriles including 3-methyl-2-butenenitrile and 3-methyl-2-petenenitrile gave high to moderate yields of 90% and 60%, respectively.[15]

On the other hand, we demonstrate that aromatic substrates give yields ranging from 12% to 28%. The aliphatic alkyl substrates exhibited the highest yields of all compounds tested with **15** giving a 36% yield and **16** produced in 31% yield. This of course is an improvement on the NaH reaction, which in these instances gave no product. However, the reverse was observed in the case of small aromatics where product yields were low with LDA as opposed to NaH. For instance, thiophene (**8**) gave a yield of 28% with LDA, while the NaH promoted reaction gave an overall yield of 89%. The effect was more pronounced with the bulky substrates. Biphenyl (**13**) gave a 12% yield with LDA while a 93% yield was obtained with NaH.

The synthesized enaminonitriles, with their 1,2-relationship of the amino and nitrile groups, were subsequently exploited in the synthesis of their quinozoline derivatives via the heating in the presence of formic acid and formamide at 200 °C (Fig. 8). While it was previously reported

Table 1. Substrate effects with excess NaH as compared to LDA

R group	Compound	NaH yield (%)	LDA yield (%)
	**8**	89	28
	**9**	96	24
	**10**	88	18
	**11**	88	20
	**12**	79	20
	**13**	93	12
	**14**	90	13
	**15**	—	36
	**16**	—	31

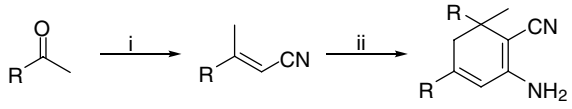


Figure 7. Reagents and conditions: (i) 1.1 equiv $C_6H_{12}NO_3P$, 1.1 equiv NaH; (ii) LDA.

that the reaction took 1 h to go to completion, we were unable to observe product formation under these conditions.[15] We, therefore, took to further optimizing this reaction. With formic acid having a low boiling point, a reflux setup was implemented. After 1 h, very little product was observed in the crude ^{1}H NMR using **8** as a model substrate. The reaction time was thus extended to 12 and 24 h, respectively. At 12 h, about half of the starting material was converted, giving a 37% yield, and at 24 h the reaction went to completion, affording a 73% yield.

Utilizing these optimized conditions, we converted each of the enaminonitriles to their corresponding quinazo-

Figure 8. Reagents and conditions: (i) CHOOH, H_2NCHO, 200 °C, 24 h.

lines (Table 2). Overall yields for each substrate were moderate with biphenyl **22** giving the highest at 78% and bromo-thiophene **18** resulting in the lowest at 60% yield. Phenyl **19** with its fluoro **20** and bromo **21** analogues gave moderate yields of 74%, 67%, and 70%, respectively. The bulky naphthalene **23** substrate was comparable to biphenyl **23** with an overall yield of 74%. The alkyl containing β-ionyl **24** and octyl **25** containing substrates were also generated in moderate yields (yet lower than the aromatics), 54% and 57%, respectively.

The biological activities of the synthesized enaminonitriles and quinazolines were evaluated in cell-based assays including their anti-proliferative/cytotoxic effects against Jurkat (a leukemic T-cell line), bacterial strains (*E. coli* and *B. subtilis*), and yeast (*S. cerevisiae*). Cytotoxicity against T-cells was measured by staining the nuclei of the cells with propidium iodide and measuring its fluorescence, while anti-microbial activity was assessed by measuring cell density at OD_{600}. The most pronounced

Table 2. Quinozoline product yields

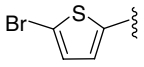
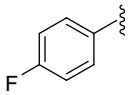
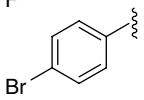
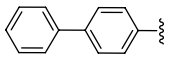
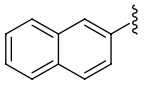
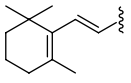
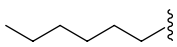
R group	Compound	Yield
	**17**	73
	**18**	60
	**19**	74
	**20**	67
	**21**	70
	**22**	78
	**23**	74
	**24**	54
	**25**	57

Table 3. Cytotoxic activities of enaminonitriles and quinazolines against Jurkat

Compound	IC_{50} (μg/ml)	μM
8	51.485	172.15
9	6.424	13.93
10	4.342	15.13
11	6.257	19.37
12	9.213	20.52
13	8.478	19.31
14	7.35	18.99
15	5.452	12.65
16	14.166	46.75
17	5.008	15.36
18	4.586	9.51
19	5.522	17.59
20	1.133	3.24
21	5.962	12.69
22	4.848	10.4
23	3.881	9.37
24	5.849	12.8
25	4.148	12.61

effects were observed against Jurkat, where in all cases the quinazolines were more active than their corresponding enaminonitriles (Table 3). The most effective compounds **20** (CC_{50} 3.24 μM), **23** (CC_{50} 9.37 μM), and **18** (CC_{50} 9.51 μM) were heterocyclic and in many instances, halogenated. With respect to the anti-microbial assays, all compounds displayed moderate activities. MIC values were $\geqslant$163 μM in *B. subtilis*, $\geqslant$189 μM in *E. coli*, and $\geqslant$197 μM against *S. cerevisiae*.

We report on a one-pot strategy in the preparation of cyclohexadiene enaminonitriles. The reaction is high yielding with heterocyclic systems and other bulky substrates thus complementing the previously reported syntheses. The cyclohexadiene enaminonitriles were additionally implemented as precursors in the synthesis of quinazolines, a scaffold with demonstrated therapeutic activities. In this regard, we optimized reaction conditions reported in the literature, which generated each quinozoline in moderate yields.

In cell-based assays, of all compounds tested, the quinazolines gave the most promising effects, where CC_{50} values in the low micromolar range were measured against a human leukemic cell-line. The identification of the cellular targets of these lead compounds might direct the synthesis of functionally and structurally related analogues with more potent biological activities.

Supplementary data including experimental details, NMR spectra, and biological data are available online free of charge.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.048.

References and notes

1. Hickmott, P. W. *Tetrahedron* **1982**, *38*, 1975.
2. Blaha, K.; Cervinka, O. *Adv. Org. Chem.* **1963**, *4*, 1.
3. Albert, A. *Adv. Heterocycl. Chem.* **1982**, *32*, 1.
4. Wamhoff, H. *Adv. Heterocycl. Chem.* **1985**, *32*, 1.
5. Wahba, A. E. *Chem. Rev.* **1993**, *93*, 1991.
6. Ashton, W. T.; Walker, F. C., Jr.; Hynes, J. B. *J. Med. Chem.* **1973**, *16*, 694.
7. Jen, T.; Dienel, B.; Bowman, H.; Petta, J.; Helt, A.; Love, B. *J. Med. Chem.* **1972**, *15*, 727.
8. Chern, J. W.; Tao, P. L.; Yen, M. H.; Lu, G. Y.; Shaiu, C. Y.; Lai, Y. J.; Chien, S. L.; Chan, C. H. *J. Med. Chem.* **1993**, *36*, 2196.
9. Chern, J. W.; Shiau, C. Y.; Lu, G. Y. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 571.
10. Campbell, S. F.; Michael, M. J.; Hardstone, J. D.; Lewis, B. N.; Palmer, M. J. *J. Med. Chem.* **1987**, *30*, 49.
11. Civantos Calzada, B.; Aleixandre de Artinano, A. *Pharmacol. Res.* **2001**, *44*, 195.
12. Docherty, J. R. *Pharmacol. Ther.* **1989**, *44*, 241.
13. Perrier, V.; Wallace, A. C.; Kaneko, K.; Safar, J.; Prusiner, S. B.; Cohen, F. E. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6073.
14. Pan, K. M.; Baldwin, M.; Nguyen, J.; Gasset, M.; Serban, A.; Groth, D.; Mehlhorn, I.; Huang, Z. W.; Fletterick, R. J.; Cohen, F. E.; Prusiner, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10962.
15. Tucker, H.; Golding, G.; Purvis, S. R. *Tetrahedron Lett.* **1981**, *38*, 1373.
16. Mirek, J.; Adamczyk, M.; Mokrosz, M. *Synthesis* **1980**, 296.
17. Shabtai, J.; Ney-Igner, E.; Pines, H. *J. Chem. Soc., Perkin I* **1973**, 2230.
18. Bench, B. J.; Liu, C.; Evett, C. R.; Watanabe, C. M. H. *J. Org. Chem.* **2006**, *71*, 9458.
19. Bench, B. J.; Tichy, S. E.; Perez, L. M.; Benson, J.; Watanabe, C. M. H., submitted for publication.
20. Asato, A. E.; Watanabe, C.; Li, X.-Y.; Liu, R. S. H. *Tetrahedron Lett.* **1992**, *33*, 3105.
21. Uchikawa, O.; Fukatsu, K.; Tokunoh, R.; Kawada, M.; Matsumoto, K.; Imai, Y.; Hinuma, S.; Kato, K.; Nishikawa, H.; Hirai, K.; Miyamoto, M.; Ohkawa, S. *J. Med. Chem.* **2002**, *45*, 4222.