Bioorganic & Medicinal Chemistry Letters 19 (2009) 6939–6942

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Synthesis and biological evaluation of 2-amino-7,7-dimethyl 4-substituted-5-oxo-1-(3,4,5-trimethoxy)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile derivatives as potential cytotoxic agents

Saleh I. Alqasoumi [a], Areej M. Al-Taweel [b], Ahmed M. Alafeefy [c], Mostafa M. Hamed [d], Eman Noaman [e], Mostafa M. Ghorab [a,*]

[a] Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia
[b] Pharmacognosy Department, of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia
[c] Medicinal Chemistry Department, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia
[d] Department of Pharmaceutical chemistry, Faculty of Pharmacy & Biotechnology, German University in Cairo, New Cairo City, Egypt
[e] Radiation Biology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt

ARTICLE INFO

*Article history:*
Received 7 August 2009
Revised 11 October 2009
Accepted 15 October 2009
Available online 20 October 2009

*Keywords:*
Hexahydroquinoline
Trimethoxyphenyl
Anticancer
Tubulin

ABSTRACT

A large number of antimitotic drugs, derived from natural sources or chemically synthesized, have been identified and shown to interfere with the tubulin system. Inhibition of tubulin polymerization is among the important targets useful in the cancer therapy.
The present work reports the synthesis of some novel quinoline derivatives bearing a trimethoxyphenyl moiety. The trimethoxybenzene moiety has been reported to be crucial to obtain relevant cytotoxic and antitubulin responses. All the newly synthesized compounds were evaluated for their in vitro anticancer activity. Several compounds showed interesting cytotoxic activities compared to the used reference drug.


In the development of compounds that can be used in anticancer chemotherapy, the mitotic spindle was found to be among the attractive targets. The mitotic spindle is formed by microtubules which are cykloskeletal polymers of tubulin involved in many cellular functions, and the compounds affecting the spindle will lead to the accumulation of cells arrested in metaphase.[1–4] Several antimitotic drugs with diverse structures, derived from natural sources or obtained by screening compound libraries, have been identified and shown to affect the tubulin system.[4–6]

One of the most important tubulin-binding agents is combretastatin A-4 (**I**), isolated from the bark of the South African tree *Combretum caffrum*,[7] strongly inhibits the polymerization of tubulin by binding to the colchicine site.[8] This compound had particular interest not only for its anticancer activity but also because it has been from the simplest natural compounds with potent antitubulin activity,[8] and so SAR studies have been done to evaluate a large number of developed combretastatin A-4 analogues.[9] Among synthetic small-molecule tubulin inhibitors, was the CA-4 analogue named phenstatin (**II**). Recently a series of thiophene derivatives (**III**) have been synthesized and were found to be potent inhibitors of tubulin polymerization.[10] Romagnoli et al. have then synthesized a series of benzo[*b*]thiophene (**IV**) and tetrahydro[2,3-*c*]pyridine (**V**) derivatives showing interesting activities in inhibiting microtubule polymerization and cell proliferation (Fig. 1).[4,11]

In addition, quinoline derivatives have received considerable attention because of their pivotal role in various biological processes and numerous derivatives of quinolines have been reported to have wide biological activities including the anticancer activity.[12–16] Therefore, in a search for novel cytotoxic agents with an expected antimitotic activity, we prompted to synthesize a new series of hexahydroquinoline derivatives bearing a trimethoxyphenyl moiety. This trimethoxyphenyl moiety has been reported to be crucial to obtain relevant cytotoxic and antitubulin potency for the series of molecules occupying the colchicines-binding site in the tubulin protein.[4,9,17] All the newly synthesized compounds were evaluated for their in vitro anticancer activity against the used reference drug.

The present work reports the possible utility of 3-(3,4,5-trimethoxyanilino)-5,5-dimethyl-cyclohex-2-enone **3** in the synthesis of 2-amino-7,7-dimethyl-5-oxo-4-substituted-aryl-1-(3,4,5-trimethoxyphenyl)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitriles **6a–t**.

* Corresponding author. Tel.: +966 534292860; fax: +966 (01) 4670560.
*E-mail address:* mmsghorab@yahoo.com (M.M. Ghorab).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.065

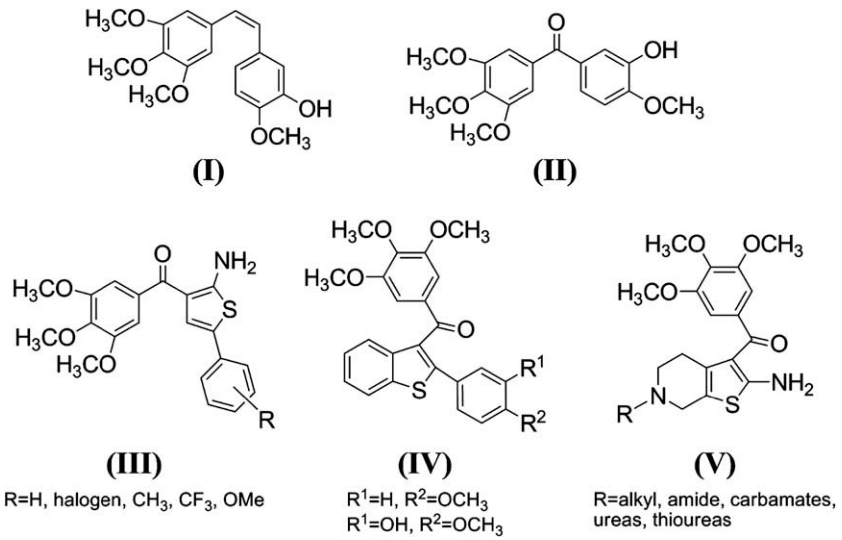



**Figure 1.** Different compounds which inhibit tubulin polymerization.

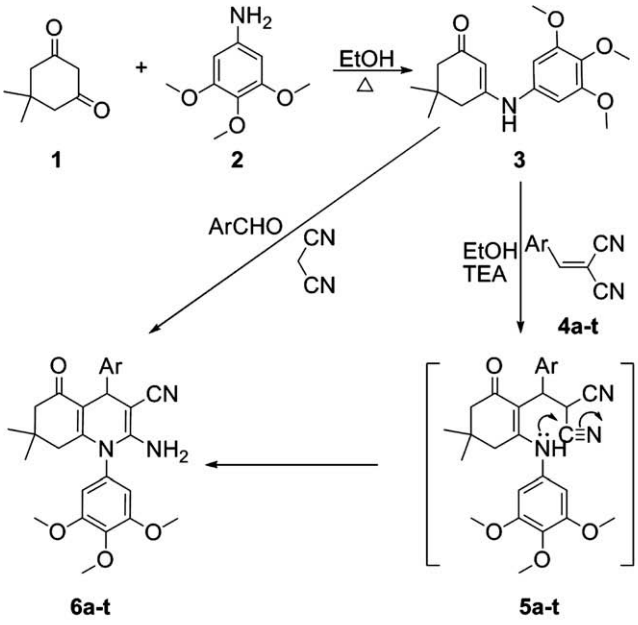
Enaminone **3** was obtained by condensation of 5,5-dimethylcyclohexan-1,3-dione **1** with 3,4,5-trimethoxyaniline **2** (Scheme 1). The structure of compound **3** was supported by elemental analysis and spectral data. IR spectrum of compound **3** revealed the presence of bands for NH at 3270 $cm^{-1}$, for (CH aliph.) at 2955, 2930, 2824 $cm^{-1}$ and for C=O at 1620 $cm^{-1}$. Also, $^1H$ NMR spectrum (in DMSO-$d_6$), indicated the presence of a signal at 8.7 ppm which could be assigned to NH of enaminone **3** and 3.6, 3.71, 3.79 ppm for the three methoxy groups.

We have studied the reaction of activated cyano olefins **4a–t** with enaminone **3** as a route to functionally substituted quinolines. Condensation of **3** with **4a–t** in a molar ratio (1:1) in refluxing ethanol containing triethylamine afforded the hexahydroquinoline derivatives **6a–t**. Formation of compounds, **6a–t**, could be explained via initial Michael addition of enaminone **3** to the ylidenic bond in **4a–t** forming an acyclic intermediate **5a–t** which cyclizes by nucleophilic attack of the NH group on the cyano carbon, followed by tautomerisation to the final products **6a–t**.

Compounds **6a–t** were unambiguously synthesized by another route involving one-pot condensation of the appropriate aldehydes, malononitrile and enaminone **3** in a molar ratio (1:1:1) in refluxing ethanol containing triethyamine (TEA) as catalyst. In this case, formation of **6a–t** are illustrated in terms of initial condensation of the aldehyde with malononitrile affording the activated cyano olefin **4a–t**, followed by addition of the enaminone **3** to arylidenemalononitrile **4a–t** as above.

The *N*-aryl substituent, 3,4,5-trimethoxyphenyl, promoted the nucleophilicity of the enaminone **3** towards 2-arylidenemalononitrile **4a–t**. Compound **3** nucleophilically adds to the arylidenemalononitrile **4a–t** to form an intermediate iminium ion that is deprotonated by TEA (Scheme 2). The structure of compounds **6a–t** was established on the basis of analytical and spectral data. Thus, IR spectra of compounds **6a–t** showed bands at 3469–3206 $cm^{-1}$ due ($NH_2$), 2226–2162 $cm^{-1}$ (CN), 1668–1620 $cm^{-1}$ (C=O).

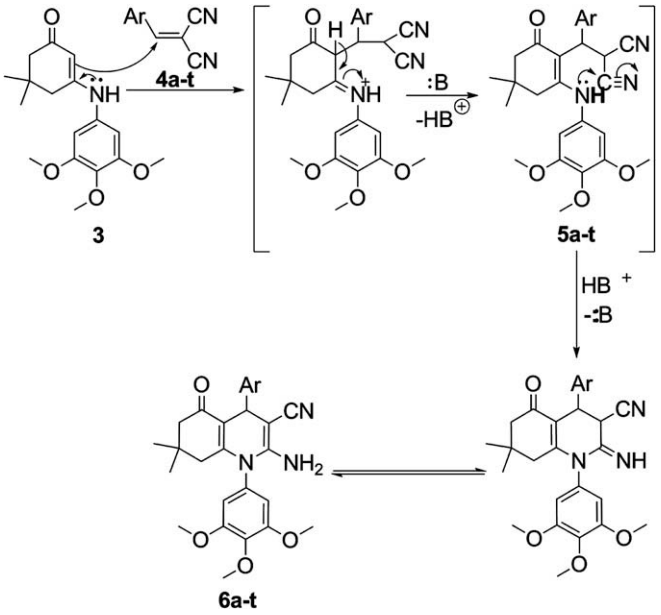
Doxorubicin, the reference drug used in this study is one of the most effective antitumor agents used to produce regressions in acute leukemia's Hodgkin's disease, and other lymphomas. The relationship between survival ratio and drug concentration was plotted to obtain the survival curve of Ehrlich Ascites Carcinoma (EAC) cell line. The response parameter calculated was $IC_{50}$ value (Table 1), which corresponds to the compound concentration causing 50% mortality in net cells.




**6a:** **Ar**= $C_6H_5$.
**6b:** **Ar**= 4-($C_6H_4$)$CH_3$.
**6c:** **Ar**= 2-($C_6H_4$)OH.
**6d:** **Ar**= 4-($C_6H_4$)OH.
**6e:** **Ar**= CH=CH-$C_6H_5$.
**6f:** **Ar**= 2-($C_6H_4$)$OCH_3$.
**6g:** **Ar**= 4-($C_6H_4$)$OCH_3$.
**6h:** **Ar**= 2-($C_6H_4$)Cl.
**6i:** **Ar**= piperonyl.
**6j:** **Ar**= 3-($C_6H_4$)$NO_2$.
**6k:** **Ar**= 4-($C_6H_4$)$NO_2$.
**6l:** **Ar**= $C_6H_3$.$OCH_3$-3, OH-4.
**6m:** **Ar**= $C_6H_3$.$Cl_2$.2,4.
**6n:** **Ar**= $C_6H_3$.$OC_2H_5$-3, $OCH_3$-4.
**6o:** Ar= $C_6H_2$.$(OCH_3)_3$.2,4,5.
**6p:** **Ar**= 2-OH-naphthyl.
**6q:** **Ar**= 2-$OCH_3$-naphthyl.
**6r:** **Ar**= 4-$OCH_3$-naphthyl.
**6s:** **Ar**= 2-furyl-5-$CH_3$.
**6t:** **Ar**= 2-thienyl.

**Scheme 1.**

All the newly synthesized compounds were evaluated for their in vitro cytotoxic activity. Compounds **6f** containing 2-methoxyphenyl at position-4 with cyano group at position-3 ($IC_{50}$ value = 26.58 μM), **6b** having 4-methylphenyl at position-4 with cyano group at position-3 ($IC_{50}$ value = 52.85 μM), and **6k** bearing 4-nitrophenyl at position-4 with cyano group at position-3 ($IC_{50}$




**Scheme 2.** Postulated mechanism for the formation of compounds **6a–t**.

value = 49.60 μM) are more potent and efficacious than the reference drug doxorubicin ((IC$_{50}$ value = 68.13 μM). Additionally compound **6d** having 4-hydroxyphenyl at position-4 with cyano group at position-3 (IC$_{50}$ value = 75.78 μM) is nearly as active as doxorubicin. On the other hand compounds **6m, 6p** and **6s** are less active than doxorubicin as positive control.

A molecular modeling study has been performed to investigate the possible binding conformation for this group of compounds to the colchicine binding site of tubulin, which may give a suggestion about their proposed mechanism of action as antitubulin agents. Compounds **6f and 6k** were docked in the (PDB entry: 1SA0)[3] using MOE 2007.09 (MOE).[18]

**Table 1**
In vitro cytotoxic activity of the newly synthesized compounds **6a–t**

| Compound | Non-viable cells (%) | | | | IC$_{50}$[a] (μg/mL) | IC$_{50}$[a] (μM) |
|---|---|---|---|---|---|---|
| | Concentration (μg mL$^{-1}$) | | | | | |
| | 100 | 50 | 25 | 10 | | |
| Doxorubicin | 100 | 68 | 30 | 24 | 37 | 68.13 |
| **6a** | 10 | 8 | 6 | 0 | >100* | – |
| **6b** | 90 | 60 | 50 | 30 | 25 | 52.85 |
| **6c** | 10 | 2 | 0 | 0 | >100* | – |
| **6d** | 100 | 90 | 20 | 10 | 36 | 75.78 |
| **6e** | 40 | 30 | 30 | 10 | >100* | – |
| **6f** | 100 | 70 | 65 | 30 | 13 | 26.58 |
| **6g** | 15 | 11 | 1 | 0 | >100* | – |
| **6h** | 20 | 20 | 15 | 10 | >100* | – |
| **6i** | 40 | 40 | 30 | 10 | >100* | – |
| **6j** | 30 | 30 | 20 | 5 | >100* | – |
| **6k** | 80 | 70 | 50 | 10 | 25 | 49.60 |
| **6l** | 40 | 30 | 20 | 10 | >100* | – |
| **6m** | 100 | 95 | 55 | 20 | 50 | 94.69 |
| **6n** | 45 | 40 | 10 | 2 | >100* | – |
| **6o** | 40 | 30 | 30 | 20 | >100* | – |
| **6p** | 90 | 15 | 10 | 2 | 75 | 142.85 |
| **6q** | 30 | 10 | 5 | 0 | >100* | – |
| **6r** | 40 | 40 | 30 | 10 | >100* | – |
| **6s** | 50 | 41 | 31 | 21 | 100 | 215.98 |
| **6t** | 20 | 15 | 10 | 5 | >100* | – |

[a] IC$_{50}$ value: corresponds to the compound concentration causing 50% mortality in net cells.
* Compounds with IC$_{50}$ >100 μg/mL are considered to be inactive.

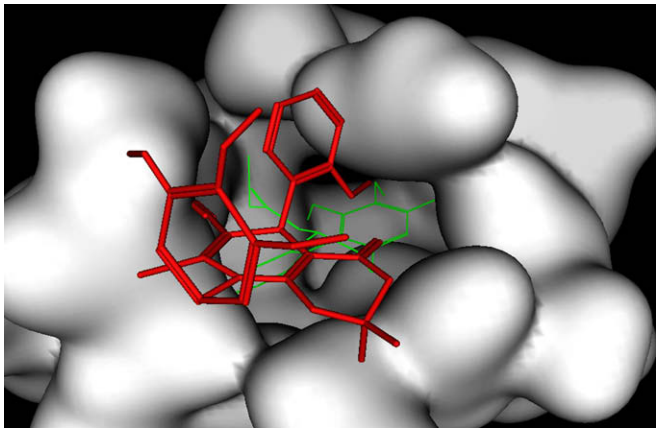


**Figure 2.** Docking pose of compound **6f** 'red' in the colchicine site. DAMA-colchicine in green.

In order to validate our docking procedure, we docked the DAMA-colchicine which is the ligand already co-crystallised with the protein. The docking results showed that the best scored conformation exhibits a similar fashion as the crystallized conformation with an RMSD of 1.1 Å.

We then docked the most active compounds, **6f** and **6k**, and in both cases, it was found that the trimethoxyphenyl moiety is positioned in a different place when compared to the corresponding colchicinoid A ring, and even the whole molecule exhibits totally different conformation than the colchicine analogue in the crystallized protein complex. (Figs. 2 and 3)

From the figures, it is clear that the compounds showing significant cytotoxic activity exhibits a different conformation and binding mode, in the colchicine binding site of tubulin, compared to the DAMA-colchicine. Therefore, it could be suggested that these compounds may exert their cytotoxic activity by a different mechanism of action other than being antimitotic agents through the inhibition of the tubulin polymerization.

In conclusion, the objective of the present study was to synthesize and investigate the anticancer activity of new hybrid compounds comprising hexahydroquinolines, bearing cyano group at position-3 with different moieties at position-4 and 3,4,5-trimethoxyphenyl at position-1, for synergistic purpose. The results revealed that the corresponding quinoline derivatives **6f, 6b** and **6k** showed the highest in vitro cytotoxic activity when compared to other tested compounds and doxorubicin as a reference drug.

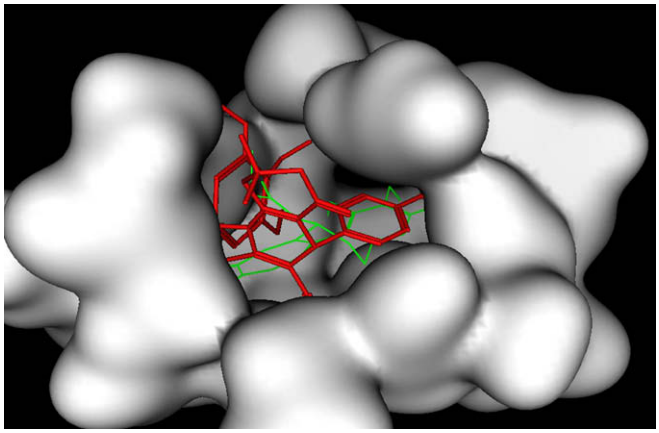


**Figure 3.** Docking pose of compound **6k** 'red' in the colchicine site. DAMA-colchicine in green.

Additionally, compound **6d** is nearly as active as doxorubicin. Docking of the most active compounds, in the colchicine binding site of tubulin, may give a suggestion that these compounds may exert their cytotoxic activity by a different mechanism of action other than being antimitotic agents through the inhibition of the tubulin polymerization.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.065.

## References and notes


1. Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253.
2. Pasquier, E.; Andre, N.; Braguer, D. *Curr. Cancer Drug Targets* **2007**, *7*, 566.
3. Ravelli, R. B.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198.
4. Romagnoli, R.; Baraldi, P. G.; Carrion, M. D.; Cruz-Lopez, O.; Cara, C. L.; Tolomeo, M.; Grimaudo, S.; Di Cristina, A.; Pipitone, M. R.; Balzarini, J.; Kandil, S.; Brancale, A.; Sarkar, T.; Hamel, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5041.
5. Attard, G.; Greystoke, A.; Kaye, S.; De Bono, J. *Pathol. Biol. (Paris)* **2006**, *54*, 72.
6. Honore, S.; Pasquier, E.; Braguer, D. *Cell Mol. Life Sci.* **2005**, *62*, 3039.
7. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
8. Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984.
9. Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033.
10. Romagnoli, R.; Baraldi, P. G.; Pavani, M. G.; Tabrizi, M. A.; Preti, D.; Fruttarolo, F.; Piccagli, L.; Jung, M. K.; Hamel, E.; Borgatti, M.; Gambari, R. *J. Med. Chem.* **2006**, *49*, 3906.
11. Romagnoli, R.; Baraldi, P. G.; Carrion, M. D.; Lopez Cara, C.; Preti, D.; Fruttarolo, F.; Pavani, M. G.; Tabrizi, M. A.; Tolomeo, M.; Grimaudo, S.; Di Cristina, A.; Balzarini, J.; Hadfield, J. A.; Brancale, A.; Hamel, E. *J. Med. Chem.* **2007**, *50*, 2273.
12. Chen, Y. L.; Huang, C. J.; Huang, Z. Y.; Tseng, C. H.; Chang, F. S.; Yang, S. H.; Lin, S. R.; Tzeng, C. C. *Bioorg. Med. Chem.* **2006**, *14*, 3098.
13. Ghorab, M. M.; Ragab, F. A.; Hamed, M. M. *Eur. J. Med. Chem.* **2009**, *44*, 4211.
14. Gopal, M.; Shenoy, S.; Doddamani, L. S. *J. Photochem. Photobiol. B* **2003**, *72*, 69.
15. Kim, Y. H.; Shin, K. J.; Lee, T. G.; Kim, E.; Lee, M. S.; Ryu, S. H.; Suh, P. G. *Biochem. Pharmacol.* **2005**, *69*, 1333.
16. Zhao, Y. L.; Chen, Y. L.; Chang, F. S.; Tzeng, C. C. *Eur. J. Med. Chem.* **2005**, *40*, 792.
17. Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H. M.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 2293.
18. Molecular Operating Environment (MOE 2007.09). Chemical Computing Group, Inc. Montreal, Quebec, Canada.