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# Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and Evaluation of Their Antiproliferative Activity

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## Graphical Abstract

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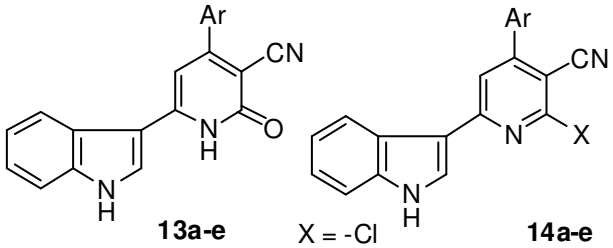


**13a-e**

X = -Cl **14a-e**

X = -NH(CH)$_2$NH$_2$ **15a-e**

# Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and evaluation of their antiproliferative activity

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ABSTRACT

A novel class of 6-indolypyridine-3-carbonitrilile derivatives were synthesized and evaluated for antiproliferative activities to establish structure-activity relationship. The synthesis was carried out through one-pot multicomponent reaction of 3-acetylindole, aromatic aldehydes, ethyl cyanoacetate, and ammonium acetate in the presence of piperidine as a catalyst, using a microwave irradiation method or a traditional thermal method. This was followed by chlorination for compounds **13a-e** and subsequent nucleophilic substitution of the chlorine group by ethylenediamine at $C_2$ position of the pyridine ring. The antiproliferative activity of these new nicotinonitriles was evaluated against human ovarian adenocarcinoma (SK-OV-3), breast adenocarcinoma (MCF-7), and cervix adenocarcinoma (HeLa) cells. Among all compounds, 2-((2-aminoethyl)amino)-4-aryl-6-indolylnicotinonitriles series (**15a**, **15b**, **15d**, and **15e**) exhibited higher antiproliferative activity cells with $IC_{50}$ values of 4.1-13.4 µM.



3-Substitued indolyl moiety is a basic constituent in numerous proteins, the neurotransmitter serotonin, and mammalian hormone melatonin as well as a large number of marketed available pharmaceutical drugs. For instance, indomethacin (**1**), naratriptan (**2**), tegaserod (**3**), ondansetron (**4**), zafirlukast (**5**), sunitinib (**6**), sertinole (**7**), and panobinostat (**8**) (Figure 1) contain 3-substituted indole scaffold in their chemical structures.[1]

Additionally, several marine indole alkaloids have been isolated and evaluated for their anticancer, antiviral, and antiinflammatory activities. Meridianins A-E (**9**) were isolated from tunicate *Aplidium Meridianm*.[2,3] Bisindolyl alkaloids spaced by five or six membered heterocyclic moieties, such as piperazinone (Hamacanthin B, **10**), quinone (Asterriquinone, **11**), or imidazole (Nortopsentins A-C, **12**) (Figure 1) have exhibited modest to high anticancer activities against a wide range of human cell lines at micromolar concentrations.[4-6]

Furthermore, nicotinonitrile skeletons especially those with amino substituent at $C_2$ and/or 4,6-diaryl-substitutent have demonstrated broad range of biological activities, such as antibacterial,[8], antifungal[9], antituberculosis[10], antiviral[11], antipyretic, analgesic, and antiinflammatory[12-14] effects.

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Furthermore, they have been used as inhibitors for protein kinase, topoisomerase[15,16], phosphodiesterase[17,18] as well as antiproliferative agents for the treatment of a number of human cancer cell lines.[19-21]

The application of one-pot multicomponent reactions (MCRs) and microwave-assisted have been demonstrated to offer smooth reaction conditions and higher overall yield when compared to classical synthesis methodologies.[22-28] Bis(3′-indolyl)pyridine and pyrazolopyridine-indolyl derivatives have been previously synthesized through MCR and/or microwave assisted reactions[29a.b] according to the previously reported procedure[29c].

In continuation of our efforts to synthesize and evaluate new indole derivatives as antiproliferative agents,[30] we designed novel indole-3-cyanopyridine hybrid structures to determine the substituent effects at $C_2$ and $C_4$ on the cytotoxic potency of this scaffold. Although other heterocyclic indolyl derivatives have been previously synthesized,[29] To the best of our knowledge this is the first microwave-assisted synthesis of hybrid indole and 3-cyano-4-arylsubstituted pyridine compounds and evaluation of their antiproliferative activities.

Considering the advantages of MCRs approach and microwave irradiation, 3-acetyl indole ketone reacted with ethyl cyanoacetate and a series of aromatic aldehydes with an excess of ammonium acetate under microwave irradiation for 15-20 min affording novel 4-aryl-6-indolyl-nicotinonitrile-2-one derivatives (**13a-e**) (Scheme 1). All compounds were characterized by mass, and NMR spectroscopy (Supplementary Material).

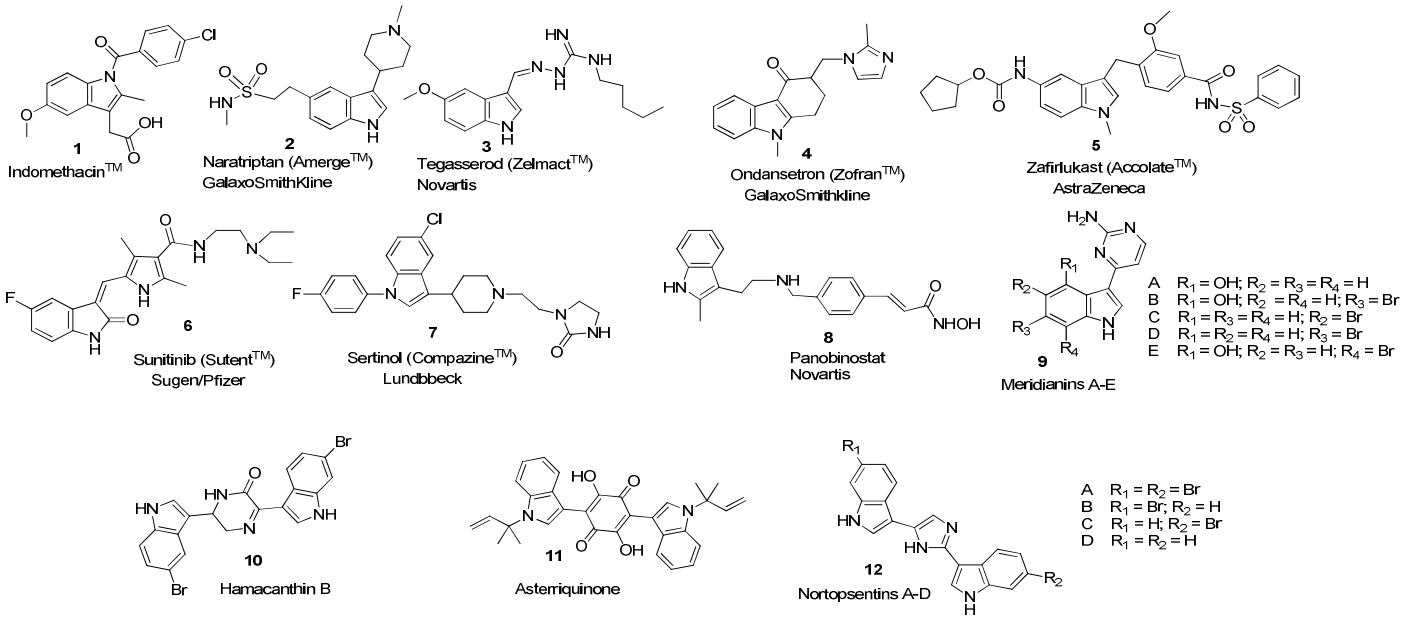
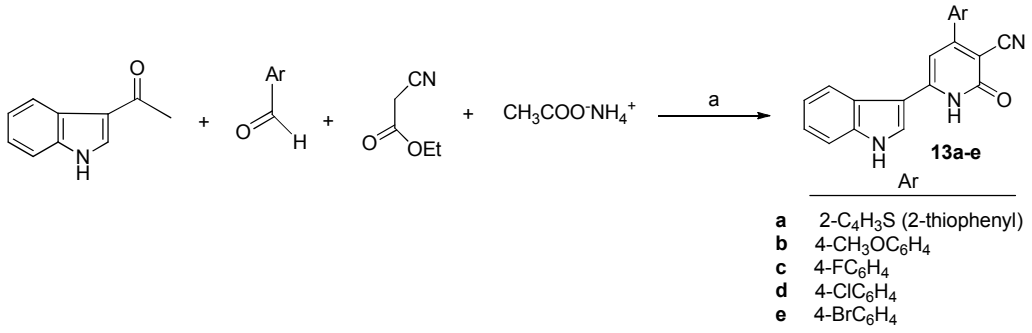
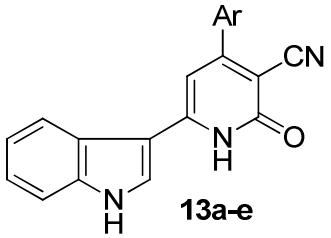



**Figure 1.** Common biologically active 3-substituted indolyl derivatives.



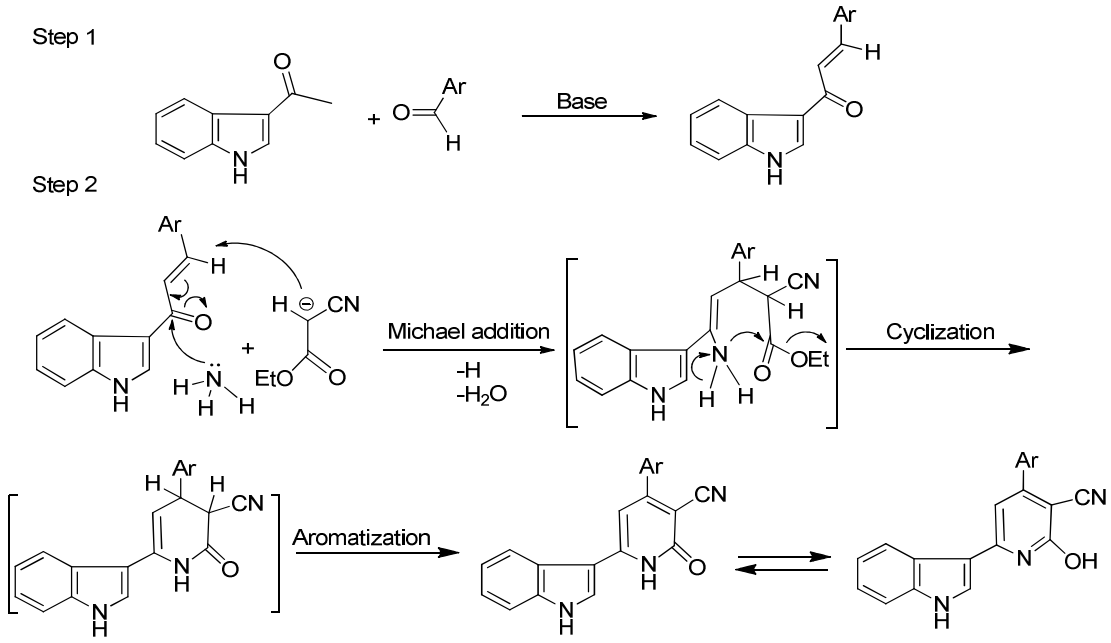

**Scheme 1**. Synthesis of indolylnicotinonitriles (**13a-e**). Reagents and conditions: (a) piperidine (1 mL), ethylene glycol (1 mL), MW irradiation (W 250 and T 150 °C).

**Table 1**. Comparative synthesis of 2-oxo-1,2-dihydropyridine-3-carbonitrile derivatives (**13a-e)** by microwave irradiation and thermal heating.




| product | Ar | Time | | Yield[c] (%) | | mp (°C) |
|---|---|---|---|---|---|---|
| | | MW[a] (min) | Th[b] (h) | MW[a] | Th[b] | |
| **13a** | 2-$C_4H_3S$ | 20 | 17 | 77 | 44 | >300 |
| **13b** | 4-$OCH_3C_6H_4$ | 20 | 18 | 79 | 45 | >300 |
| **13c** | 4-$FC_6H_4$ | 15 | 10 | 87 | 56 | >300 |
| **13d** | 4-$ClC_6H_4$ | 17 | 15 | 82 | 63 | >300 |
| **13e** | 4-$BrC_6H_4$ | 17 | 14 | 83 | 61 | >300 |

[a]The reaction was carried out by microwave irradiation at 250 W and 150 °C; [b]The reaction was carried out by thermal heating at 150 °C in oil bath; [c]Isolated yields.



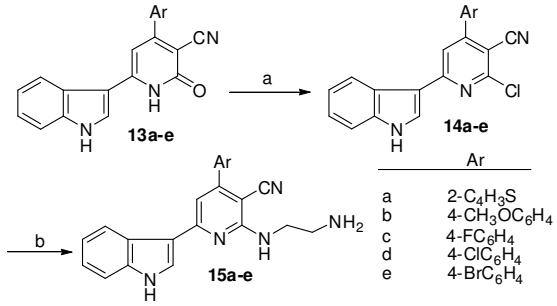

**Scheme 2**. Mechanistic illustration for the formation of 2-oxo-1,2-dihydropyridine-3-carbonitrile system.

Ethylene glycol and piperidine were used as a solvent and a catalyst, respectively, during the microwave reaction at a power of 250 W and 150 °C for a given time. The reactions were successful in achieving the benefits of both utilizing microwave irradiation and the one-pot MCRs. Compared to the traditional thermal method, the reaction time was shortened from hours to minutes with improvement in both the target product purity and overall product yield (77-87%) in case of microwave method. The results for each entry are summarized in (Table 1).

The mechanism of one pot syntheses of nicotinonitrile derivatives is known to be through the formation of α,β-unsaturated ketones intermediate via Claisen-Schimdt reaction between active methylene containing ketones and aromatic aldehydes using catalytic amount (10%) of strong bases like sodium hydroxide, triethylamine, or piperidine. This reaction is followed by condensation with nitrile containing active methylene compounds (e.g. ethyl cyanoacetate or malononitrile) through Michael addition reaction in the presence of ammonium acetate, cyclization, and aromatization to afford the corresponding 4-aryl-2-oxo-1*H*-pyridine-3-carbonitrile derivatives[31] (Scheme 2).

Results in Table 1 showed that, the electronic effect and the nature of the substituent on the aromatic aldehyde ring played a critical effect in terms of reaction time and product yield under smilar reaction conditions. When aromatic aldehydes bearing a strong electron withdrawing group (e.g. 4-fluorine, 4-chloro, 4-bromo) in *para* positions was used, the yield of the products was increased in a shorter reaction time compared to those carrying electron donating groups (e.g. 4-methoxy group) in *para* position under a similar reaction condition.

Moreover, the reaction of compounds **13a-e** with phosphoryl chloride for 18-24 h afforded the corresponding 2-chloropyridine derivatives (**14a-e**) after thermal heating at 80 °C as shown in Scheme 3. 2-Chloropyridine derivatives (**14a-e**) were used as precursors for nucleophilic substitution reaction with ethylenediamine under a reflux condition in ethanol to afford the corresponding 2-aminoethylenamino 6-indolylnicotinonitrile derivatives (**15a-e**), The chemical structures of these novel compounds **14a-e** and **15a-e** were elucidated by IR, mass, and NMR spectroscopy (see Supplementary Material).




**Scheme 3**. Reagents and conditions: (a) $POCl_3$, reflux, 80 °C for 18-24 h; (b) ethylenediamine, ethanol, reflux, 36-48 h.

The antiproliferative activities of all synthesized compounds in a panel of cancer cell lines including human ovarian adenocarcinoma (SK-OV-3), breast adenocarcinoma (MCF-7), and cervix adenocarcinoma (HeLa) cells were evaluated. All compounds (50 μM) were tested for their anticancer potency after 72 h incubation. DMSO (3%) and doxorubicin (Dox 10 μM) were used as negative and positive controls for the assay.

As it is shown in Figure 2, compounds **13a**, **13c**, **13d**, **13e**, **14a**, **14c**, and **14d** did not show any significant antiproliferative activity against HeLa, SK-OV-3, and MCF-7 cells. Among all derivatives, compounds **13b**, **14b**, and **15a-e** showed modest to high antiproliferative potency. However, compounds **15b**, **15d**, and **15e** showed comparable potency with that of Dox in HeLa cells and significantly higher potency in SK-OV-3 and MCF-7 cells versus Dox. For example, compounds **15b**, **15d**, and **15e** inhibited the proliferation of HeLa, SK-OV, and MCF-7 cells by 62-67%, 85-88%, and 84-87%. Interestingly, these three compounds inhibited the cell proliferation of SK-OV-3 and MCF-7 cells with higher potency compared to that of HeLa cells, indicating that their activity was cell-specific.

All synthesized compounds have a common scaffold of conjugated substituted 6-indolyl pyridine ring. Compounds **15a-e** also have an ethylene-1,2-diamine moiety attached to the substituted pyridine ring. Changing the substation at $C_2$ from oxo (compounds **13a-e**) to ethylene-1,2-diamine (compounds **15a-e)**

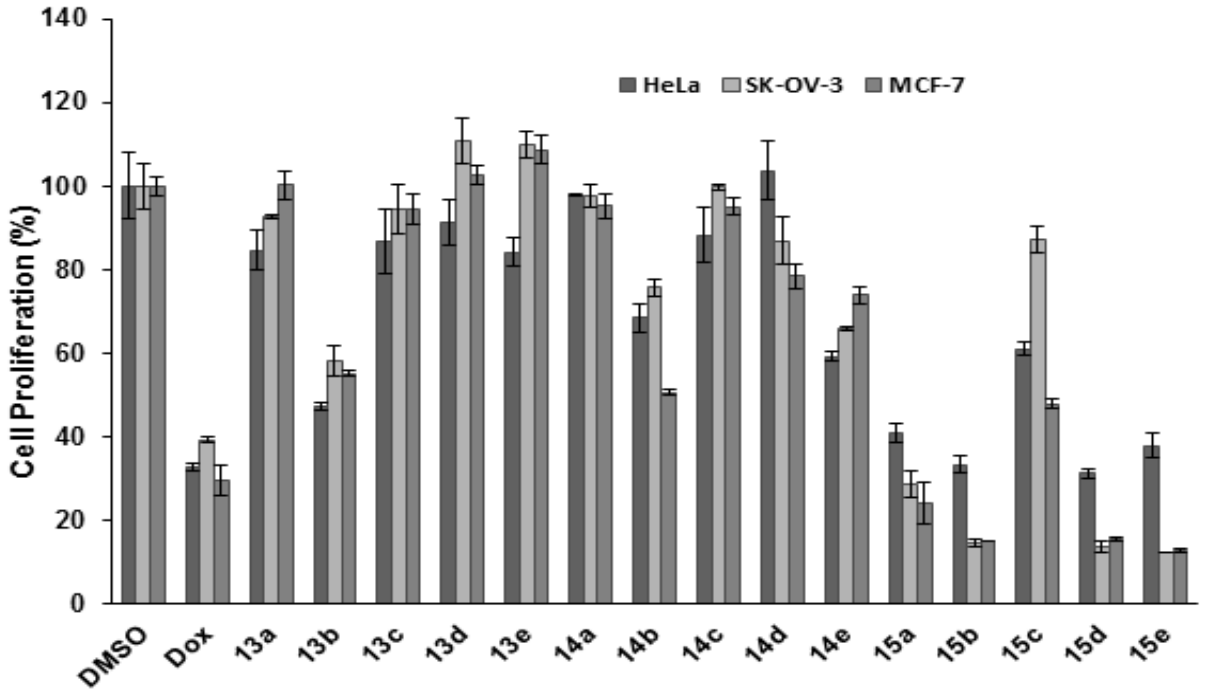



**Figure 2.** Antiproliferative activity of **13a-e**, **14a-e**, and **15a-e**.

showed that, an ethylene-1,2-diamine moiety plays a significant role in elevating the anti-proliferative activity. However, among indolyl nicotinonitrile (**15a-e**), compound **15c** with *p*-fluorophenyl substituent at $C_4$ did not show similar potency when compared with the other compounds in this series, suggesting that the presence of a strong electron withdrawing fluorine group is not productive. On the other hand, the presence of a heterocyclic ring as in compound **15a** or an electron donating group like *p*-methoxy group as in compound **15b** resulted in higher antiproliferative activity. Thus, electronic effect of the substituent of the phenyl group substituent appears to have a direct effect on antiproliferative activity.

**Table 2**. $IC_{50}$ values of four selected compounds in SK-OV-3, MCF-7, and HeLa cells.

| Entry | HeLa[a] | SK-OV-3 | MCF-7 |
|---|---|---|---|
| **15a** | 13.4 | 4.7 | 4.1 |
| **15b** | 7.2 | 6.5 | 8.1 |
| **15d** | 6.8 | 5.9 | 7.1 |
| **15e** | 8.8 | 5.8 | 6.8 |
| **Dox** | 0.15[33a] | 3.2 | 7.5[33b] |

[a]The $IC_{50}$ values of compounds were calculated in μM. The data are average of triplicate experiments.

Based on the results from the preliminary screening, compounds **15a**, **15b**, **15d**, and **15e** were selected for further $IC_{50}$ evaluation. $IC_{50}$ is the concentration that causes 50% inhibition of cancer cell growth. The $IC_{50}$ values of **15a**, **15b**, **15d**, and **15e** derivatives were tested in HeLa, SK-OV-3, and MCF-7 cells (Table 2). As it is shown in the $IC_{50}$ graphs (Figure S1, Supplementary Material), all these four derivatives showed high potency in the inhibition of the proliferation of different cancer cells. The $IC_{50}$ values of compounds **15a**, **15b**, **15d**, and **15e** were in the range of 4.1-13.4 μM, 6.5-8.1 μM, 5.9-7.1 μM, and 5.8-8.8 μM, respectively, in HeLa, SK-OV-3, and MCF-7 cells. Compounds **15a** and **15e** showed slightly lower $IC_{50}$ values in MCF-7 and SK-OV-3 cells compared to the other compounds. The partition coefficient (Log P) of all the synthesized compounds were calculated by using ChemDraw 10.0 (Supplementary information, Table S1). The data revealed that the compounds **15a-e** with moderate Log P values of 3.23-4.19 showed significantly higher antiproliferative activity compared to other compounds possibly because of higher cellular uptake of these compounds).[32] Compounds **13a-e** with low Log P values (2.43-3.28) did not show high antiproliferative activity, while compounds **14a-e** with high lipophilicity (Log P = 4.82-5.77) showed moderate activity. These data indicate that there is a correlation between the partition coefficient and antiproliferative activity of these compounds, and an optimal Log P is required for generating maximum activity.

In conclusion, we have demonstrated a facile and efficient method for the preparation of a new series of 6-indolypyridine-3-carbonitrile derivatives *via* the one-pot MCR with the microwave-assisted irradiation affording high yields, short reaction times, and the easy workup procedure. Among all compounds, 2-((2-aminoethyl)amino)-4-aryl-6-indolylnicotinonitrile series (**15a**, **15b**, **15d**, and **15e**) exhibited higher antiproliferative activity than Dox against SK-OV-3, MCF-7, and HeLa cells. These data suggest that indolylnicotinonitriles chemical scaffold can be used as a template for further structure optimization for generating compounds with higher antiproliferative activity.

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## References and notes


1. Wu, Y.-J. *Top Heterocycl. Chem*. **2010**, *26*, 1–29, Springer-Verlag Berlin Heidelberg.
2. Franco, L. H.; Joffe, E. B.K.; Puricelli, L.; Tatian, M.; Seldes, Palermo, A. M.; J.A. *J. Nat. Prod.* **1998**, *61*, 1130-2.
3. Radwan, M. A. A.; El-Sherbiny, M. *Bioorg. Med. Chem*. 2007, 14, 1206-11.
4. Sakemi, S.; Sun, H. H.; *J. Org. Chem.,* **1991**, *56*, 4304-7.
5. Gunasekera, S. P.; McCarthy, P. J.; Borges, M. K. *J. Nat. Prod.* 1994, 57, 1437-41.
6. Wijeratne, E. M. K.; Turbyville, T. J; Zhang, Z.; Bigelow, D.; Pierson, L. S.; VanEtten, H. D.; Whitesell, L.; Canfield, L. M.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2003**, *66*, 1567-73.
7. Bao, B.; Sun, Q.; Yao, X.; Hong, J.; Lee, C.O.; Sim, C. J.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2005**, *68*, 711-5.
8. Khidre, R.E.; Abu-Hashem, A. A.; El-Shazly, M. *Eur. J. Med. Chem*. **2011**, *46*, 5057-64.
9. Gholap, A. R.; Toti, K. S.; Shirazi, F. R.; Kumari, M. K.; Bhat, M. V. Deshpande; Srinivasan, K. V. *Bioorg. Med. Chem*. **2007**, *15*, 6705-15.
10. Manikannan, R.; Muthusubramanian, S.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2010**, *20,* 3352-5.
11. Ibrahim, E. S.; Elgemeie, G. E. H.; Abbasi, M. M.; Abbas, Y. A.; Elbadawi, M. A.; Attia, A. M. E. *Nucleosides and Nucleotides* **1995**, *14*, 1415-23.
12. Manna, F.; Chimenti, F.; Bolasco, A.; Filippelli, A.; Palla, A.; Filippelli, W. Lampa, E.; Mercantin, R. *Eur. J. Med. Chem*. **1992**, *27,* 627-32.
13. Hamdy, N. A.; Gamal-Eldeen A. M. *Eur. J. Med. Chem.* **2009**, *44*, 4547-56.
14. Murata, T.; Shimada, M.; Sakakibara, S.; Yoshino, T.; Masuda, T.; Shintani, T.; Sato, H.; Koriyama, Y.; Fukushima, K.; Nunami, N.; Yamauchi, Fuchikami, M.; Komura, K. H.; Watanabe, A.; Ziegelbauer, K. B.; Bacon, K. B.; Lowinger, T. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4019-22.
15. Zhao, L.-X.; Moon, Y.-S.; Kim, A. B., E. k.; Jahng, Y.; Park, J. G.; Jeong, T. C.; Cho, W.-J.; Choi, S. U.; Lee, C. O.; Lee, S.Y.; Lee, C. S.; Lee, E. S.; *Bioorg. Med. Chem. Lett*. **2004**, *14*, 1333-7.
16. Thapa, P.; Choi, R. K.; Choi, H. J. H.; Yun, M.; Jeong, B.-S.; Jung, M. J.; Nam, J. M.; Na, Y.; Cho, W.J.; Kwon, Y.; Lee, E.S. *Bioorg. Med. Chem.* **2010**, *18*, 2245-54.
17. Abadi, A. H.; Ibrahim, T. M.; Abouzid, K. M.; Lehmann, J.; Tinsley, H. N.; Gary, B. D.; Piazza, G. A. *Bioorg. Med. Chem*. **2009**, *17*, 5974-82.
18. Serry, A. M.; Luik, S.; Laufer, S.; Abadi, A. H. *J. Comb. Chem*. **2010**, *12*, 559-65.
19. Onnis, V.; Cocco, M. T.; Fadda, R.; Congiu, C.; *Bioorg. Med. Chem*. **2009**, *17*, 6158-65.
20. Fan Zhang, Yanfang Zhao, Li Sun, Lu Ding, Yucheng Gu, Ping Gong, Synthesis and anti-tumor activity of 2-amino-3-cyano-6-(1H-indol-3-yl)-4-phenylpyridine derivatives in vitro, *Eur. J. Med. Chem.* **2011**, *46*, 3149-57.
21. Elzahabi, H. S.A. *Eur. J. Med. Chem*. **2011**, *46*, 4025-34.
22. Cotterill, I. C., Usyatinsky, A. Y.; Arnold, J. M.; Douglas S. C.; Dordick J. S.; Michels, P. C.; Khmelnitsky, Y. L. *Tet. Lett.* **1998**, *39*, 1117-20.
23. Kappe, C. O.; Dallinge, D. *Nat. Rev. Drug Discovery* **2006**, 5, 51-63.
24. Tu, S.; Li, C.; Li, G.; Cao, L.; Shao, Q.; Zhou, D.; J.,B.; Zhou, J.; Xi. M. *J. Comb. Chem.* **2007**, 9, 1144-48.
25. Feng, J.; Zhou, Song, Y.-Z.; Lv, J.-S.; Gong, G.-X.; Tu, S. *Synth. Commun*. **2009**, *39*, 1443-50.
26. Poerwono, H.; Sasaki, S.; Hattori, Y.; Higashiyama, K. *Bioorg. Med. Chem. Lett.* **2010**, 20, 2086-73.
27. Mentese, M. Y.; Bayrak, H.; Uygun, Y.; Mermer, A.; Ulker, S.; Karaoglu, S. A.; Demirbas, N. Eur. J. Med. Chem. **2013**, 67, 230-42.
28. Jordão A. K.; Novais, J.; Leal, B.; Escobar, A. C.; Júnior, H. M. d-S.; Castro, H. C.; Ferreira, V.F. *Eur. J. Med. Chem*, **2013**, 63, 196-201.
29. (a) Zhu, S.-L.; Ji, S.-J.; Su, X.-M.; Sun, C.; Liu, Y. *Tet. Lett.* **2008**, *49*, 1777-1781; (b) Zhu, S.-L.; Ji, S.-J.; Zhao, K.; Liu, Y. *Tet. Lett.* **2008**, *49*, 2578-2582; (c) El-Zahor, M. I. *Al-Azhar Bulletin of Science*, **1992**, *3*, 735-41.
30. (a) Nasrolahi Shirazi, A.; Tiwari, R. K.; Brown, A.; Mandal, D.; Sun, G.; Parang. K. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3230-4*;* (b) Rao, V. K.; Chhikara, B. S.; Nasrolahi Shirazi, A.; Tiwari, R.; Parang, K.; Kumar. A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3511-4. (c) Rao, V. K.; Chhikara, B. S.; Tiwari, R.; Nasrolahi Shirazi, A.; Parang, K.; Kumar. A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 410-4.
31. Rong, L.; Wang, H.; Shi, J.; Yang, F.; Yao, H.; Tu, S.; Sh, D. *J. Heterocycl. Chem.* **2007**, *44*, 1505-08.
32. Waring M. J., *Expert Opin. Drug Discov*. **2010**, 5, 235-248.
33. (a) Réthy, B.; Kovács, A.; Zupkó, I.; Forgo, P.; Vasas, A.; Falkay, G.; Hohmann, J. *Planta Med*. **2006**, 72, 767-70.(b) Zeng, X.; Morgenstern, R.; Nyström, AM. *Biomaterials*. **2014**, 35, 1227-39.


## Supplementary Material

Supplementary data associated with this article can be found in the online version.