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Synthesis, Topoisomerase I Inhibition and Antitumor Cytotoxicity of 2,2′:6′,2″-, 2,2′:6′,3″- and 2,2′:6′,4″-Terpyridine Derivatives

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Abstract—For the development of new anticancer agents, 2,2′:6′,2″-, 2,2′:6′,3″- and 2,2′:6′,4″-terpyridine derivatives were designed and evaluated for their topoisomerase I inhibitory activity and antitumor cytotoxicity. Structure–activity relationship studies indicated that 2,2′:6′,2″-terpyridine derivatives were highly cytotoxic toward several human tumor cell lines, whereas 2,2′:6′,3″- and 2,2′:6′,4″-terpyridine derivatives were potent topoisomerase I inhibitors.

Introduction

Terpyridine has been extensively studied as a ligand in a wide range of metal complexes[1] and DNA binding agents.[2] For example, terpyridine platinum(II) complexes have been reported to possess a strong antiprotozoal activity.[3] Recently, we have reported that terthiophene derivatives, bioisosteres of terpyridine, showed not only a considerable inhibition of protein kinase C activity but also antitumor cytotoxicity against several human tumor cell lines.[4] Since the terpyridine moiety is composed of a conjugated aromatic ring system that has a relatively planar structure, it can be expected that terpyridine derivatives may be able to interact with topoisomerase I.[5] However, neither the effects of terpyridine derivatives on topoisomerase I nor the cytotoxicity have been systematically studied.

In this study, we synthesized a series of terpyridine derivatives as bioisosteres of terthiophene, which were evaluated for their inhibitory activity on topoisomerase I and cytotoxic effects on several human tumor cell lines for the development of novel anticancer agents. The structure–activity relationship was also determined.

For the design of terpyridine derivatives, 2,2′:6′,2″-, 2,2′:6′,3″- or 2,2′:6′,4″-terpyridine moiety was utilized as a basic skeleton and phenyl, 2-furyl, 3-furyl, 2-thienyl and 3-thienyl moieties were attached to the 4′-position of the terpyridine structure (Fig. 1).

Chemistry

Synthetic methods for the preparation of terpyridine derivatives are summarized in Scheme 1. Benzaldehyde (**1a**), 2-furaldehyde (**1b**), 3-furaldehyde (**1c**), 2-thiophene carboxaldehyde (**1d**) or 3-thiophenecarboxaldehyde (**1e**) was treated with 2-acetylpyridine (**2**) in the presence of KOH in methanol/water (5:1) to afford intermediates **3a–e** in a 73.3–96.0% yield. Using modified Kröhnke synthesis,[6] final products **7a–e**, **8a–e** and **9a–e** were prepared by treatment of **3a–e** with 1-(2-oxo-2-pyridin-2-yl-ethyl)pyridinium iodide (**4**), 1-(2-oxo-2-pyridin-3-yl-ethyl)pyridinium iodide (**5**) or 1-(2-oxo-2-pyridin-4-yl-ethyl)pyridinium iodide (**6**) in the presence of ammonium acetate in methanol to give **7a–e**, **8a–e** or **9a–e** in a 57.3–78.2% yield. Pyridinium iodides **4**, **5** or **6** were

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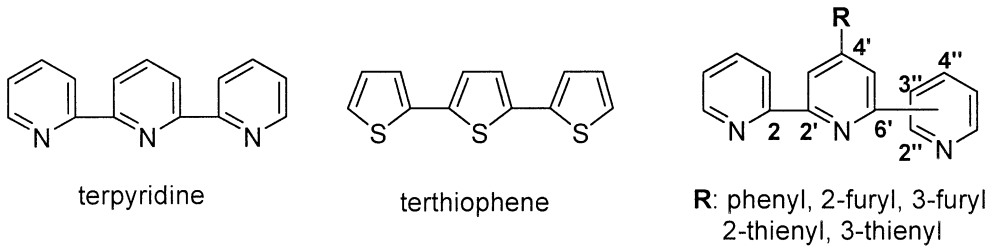
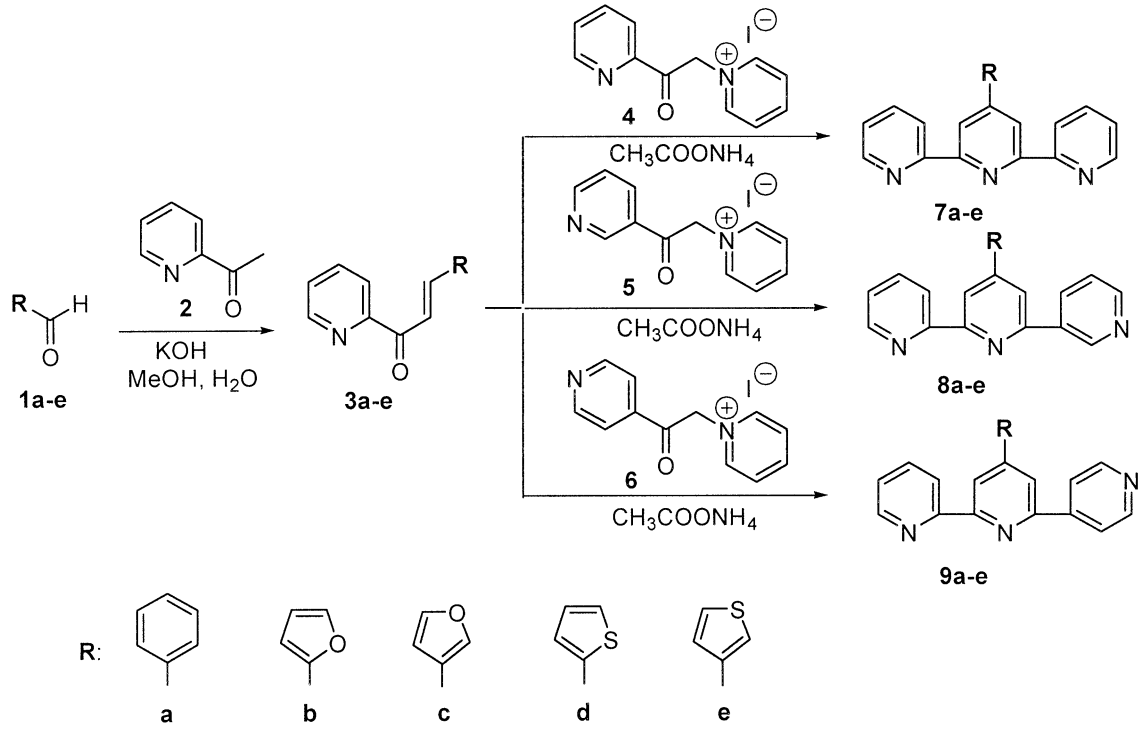


Figure 1.



Scheme 1.

prepared in a quantitative yield by treatment of 2-acetylpyridine, 3-acetylpyridine or 4-acetylpyridine with iodine in pyridine.

Results and Discussion

For the evaluation of antitumor cytotoxicity, seven different human tumor cell lines and one human normal cell line were utilized: A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma), HT-29 (human colon adenocarcinoma), A-549 (human lung carcinoma), HCT-15 (human colon adenocarcinoma), SK-OV-3 (human ovary adenocarcinoma), SK-MEL-2 (human malignant melanoma) and RPTEC (human normal renal proximal tubule epithelial cells). Many terpyridine compounds exhibited significant cytotoxicity against human tumor cell lines as summarized in Table 1. Other terpyridine derivatives with GI_{50} greater than 1.0 μg/mL against all cell lines were not shown in Table 1.

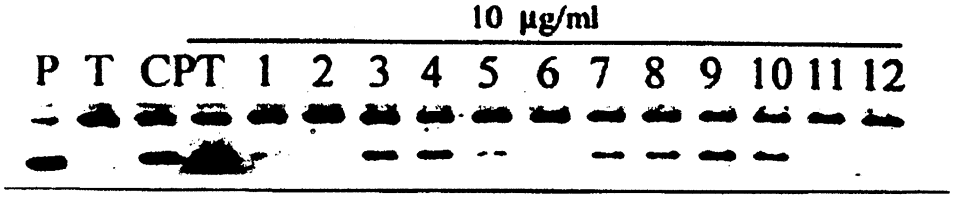
These cytotoxicity results clearly showed that α-terpyridine (2,2′:6′,2″-terpyridine) and its derivatives (**7a**–**e**) displayed stronger cytotoxicity against A-498, PC-3 and HT-29 tumor cell lines than doxorubicin. The furyl and thienyl analogues (**7b**–**e**) were relatively more cytotoxic than the corresponding phenyl analogue (**7a**). Meanwhile, it is important to notice that all α-terpyridines exhibited significantly selective renal cytotoxicity index [GI_{50}(RPTEC)/GI_{50}(A-498): 10^2–10^3]. Moreover, the 2,2′:6′,3″- or 2,2′:6′,4″-terpyridine derivatives showed considerably weaker cytotoxicity than the 2,2′:6′,2″-terpyridine derivatives, suggesting that the α-linkage for the pyridine moieties is critical to antitumor cytotoxicity.

Topoisomerase I inhibitory activities for 11 terpyridine derivatives are shown in Figure 2. Compounds **8a**, **8b**, **8d**,[10] **8e**, **9b** and **9d** exhibited strong topoisomerase I inhibitory activities. It is interesting to note that the highly toxic α-terpyridine compounds (α-terpyridine and **7b**–**e**) did not display significant topoisomerase I inhibitory activity. On the other hand, the less cytotoxic 2,2′:6′,3″- and 2,2′:6′,4″-terpyridine derivatives displayed strong topoisomerase I inhibitory activity. These results indicated that the inhibition of topoisomerase I was not the primary mechanism for the antitumor

Table 1. Antitumor cytotoxicity of terpyridine compounds

	GI_{50} (μg/mL)							
	A-498[a]	RPTEC[a]	PC-3[a]	HT-29[a]	A-549[a]	HCT-15[b]	SK-OV-3[b]	SK-MEL-2[b]
Doxorubicin	6.4×10^{-3}	7.2×10^{-2}	2.9×10^{-2}	4.6×10^{-3}	9.2×10^{-2}	7.1×10^{-2}	7.3×10^{-2}	5.9×10^{-2}
Terpyridine	9.7×10^{-5}	1.4×10^{-2}	7.3×10^{-4}	2.6×10^{-3}	9.0×10^{-2}	7.1×10^{-2}	7.2×10^{-2}	1.0×10^{-1}
7a	2.5×10^{-3}	2.7×10^{-2}	1.7×10^{-2}	1.4×10^{-3}	5.6×10^{-1}	3.7×10^{-1}	1.2×10^{-1}	7.5
7b	6.4×10^{-5}	4.1×10^{-3}	2.5×10^{-3}	2.4×10^{-3}	2.2×10^{-1}	1.0×10^{-1}	1.3×10^{-1}	9.9
7c	3.2×10^{-5}	5.8×10^{-3}	4.4×10^{-3}	2.6×10^{-3}	8.0×10^{-2}	6.0×10^{-2}	6.0×10^{-1}	3.3×10^{-1}
7d	9.7×10^{-5}	1.6×10^{-3}	2.1×10^{-3}	1.8×10^{-3}	4.4×10^{-1}	1.1×10^{-1}	1.2×10^{-1}	1.8
7e	9.7×10^{-5}	4.7×10^{-3}	1.7×10^{-3}	2.7×10^{-3}	6.2×10^{-1}	2.9×10^{-1}	1.0×10^{-1}	8.9×10^{-1}
8a	7.4×10^{-2}	3.6×10^{-1}	2.8×10^{-1}	3.3×10^{-1}	44.8	40.9	49.6	89.2
8d	2.9×10^{-3}	5.6×10^{-2}	3.1×10^{-2}	1.9×10^{-1}	78.9	56.1	52.0	77.7
8e	6.4×10^{-1}	3.1×10^{-1}	5.4×10^{-2}	3.6×10^{-1}	>100	>100	>100	>100
9d	8.4×10^{-1}	6.5×10^{-1}	2.2×10^{-1}	5.2×10^{-1}	36.2	15.1	48.3	35.2

[a]Cytotoxicity was measured by the colorimeric tetrazolium-formazan method.[7]
[b]Cytotoxicity was measured by the sulforhodamine B dye-staining method.[8]



Lane P pBR322 DNA (supercoiled form)

Lane T pBR322 DNA + calf thymus Topo I (relaxed form)

Lane CPT pBR322 DNA + calf thymus Topo I + CPT (10 μg/ml)

Lane 1-11 Prepared Compounds (10 μg/ml)

1: **7a**, 2: **7b**, 3: **8a**, 4: **8b**, 5: **7d**, 6: **7e**,

7: **8d**, 8: **8e**, 9: **9d**, 10: **9b**, 11: **7c**

Lane 12 Terpyridine (10 μg/ml)

CPT: Camptothecin Compounds **8c**, **9a**, **9c**, **9e**: not determined

Figure 2. Topoisomerase I inhibitory activities[9] of terpyridine derivatives.

cytotoxicity of terpyridine compounds. In addition, the 4′-substituent effect on the topoisomerase I inhibition was not evident.

In conclusion, we have designed an efficient synthetic route to prepare 15 terpyridine derivatives and evaluated their antitumor cytotoxicity and inhibitory activity of topoisomerase I. This was the first report of the topoisomerase I inhibitory activity of terpyridine compound. The selective cytotoxicity for the α-terpyridine analogues was highly significant. Their in vivo antitumor efficacy will be evaluated. In addition, the structure–activity relationship analysis revealed that all derivatives with the 2,2′:6′,2″-terpyridine skeleton exhibited potent antitumor cytotoxicity whereas the derivatives with 2,2′:6′,3″- or 2,2′:6′,4″-terpyridine skeleton exhibited strong topoisomerase I inhibitory activity. There was no direct correlation between antitumor cytotoxicity and topoisomerase I inhibitory activity. Clearly, further study of the molecular mechanism of action for the antitumor cytotoxicity of α-terpyridine is required.

Acknowledgements

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10. The spectral data of **7d**, **8d** and **9d**: **7d**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.74 (ddd, J = 4.8, 1.8, 0.9 Hz, 2H, pyridine H-6 & pyridine H-6″), 8.69 (s, 2H, pyridine H-3′ & H-5′), 8.64 (dt, J = 8.0, 1.0 Hz, 2H, pyridine H-3 & pyridine H-3″), 7.83 (dt, J = 7.7, 1.8 Hz, 2H, pyridine H-4 & pyridine H-4″), 7.77 (dd, J = 3.7, 1.1 Hz, 1H, thiophene H-5), 7.44 (dd, J = 5.1, 1.1 Hz, 1H, thiophene H-3), 7.36 (ddd, J = 7.5, 4.8, 1.2 Hz, 2H, pyridine H-5 & pyridine H-5″), 7.16 (dd, J = 5.1, 3.7 Hz, 1H, thiophene H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.03, 156.00, 149.09, 143.37, 141.86, 136.79, 128.23, 127.05, 125.76, 123.84, 121.28, 117.10; mp 215 °C.

8d: ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.39 (dd, J = 1.8, 0.7 Hz, 1H, pyridine H-2″), 8.73 (ddd, J = 4.8, 1.8, 0.9 Hz, 1H, pyridine H-6), 8.70 (dd, J = 4.8, 1.5 Hz, 1H, pyridine H-6″), 8.67 (d, J = 1.5 Hz, 1H, pyridine H-3′), 8.62 (dt, J = 8.0, 1.0 Hz, 1H, pyridine H-3), 8.47 (dt, J = 8.0, 1.8 Hz, 1H, pyridine H-4″), 7.94 (d, J = 1.5 Hz, 1H, pyridine H-5′), 7.87 (dt, J = 7.7, 1.8 Hz, 1H, pyridine H-4), 7.72 (dd, J = 3.7, 1.1 Hz, 1H, thiophene H-5), 7.49–7.42 (m, 2H, pyridine H-5″ & thiophene H-3), 7.36 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H, pyridine H-5), 7.18 (dd, J = 5.1, 3.7 Hz, 1H, thiophene H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.82, 155.69, 154.68, 150.05, 149.10, 148.46, 143.48, 141.40, 136.88, 134.67, 134.33, 128.41, 127.28, 125.79, 124.04, 123.48, 121.39, 116.61, 116.27; mp 172 °C.

9d: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.77 (dd, J = 4.5, 1.6 Hz, 2H, pyridine H-2″ & H-6″), 8.72 (ddd, J = 4.8, 1.8, 0.9 Hz, 1H, pyridine H-6), 8.69 (d, J = 1.5 Hz, 1H, pyridine H-3′), 8.60 (dt, J = 8.0, 1.0 Hz, 1H, pyridine H-3), 8.04 (dd, J = 4.5, 1.6 Hz, 2H, pyridine H-3″ & H-5″), 7.95 (d, J = 1.5 Hz, 1H, pyridine H-5′), 7.85 (dt, J = 7.7, 1.8 Hz, 1H, pyridine H-4), 7.69 (dd, J = 3.7, 1.1 Hz, 1H, thiophene H-5), 7.46 (dd, J = 5.1, 1.1 Hz, 1H, thiophene H-3), 7.35 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H, pyridine H-5), 7.17 (dd, J = 5.1, 3.7 Hz, 1H, thiophene H-4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.86, 155.54, 154.44, 150.43, 149.11, 146.18, 143.57, 141.23, 136.88, 128.44, 127.38, 125.87, 124.11, 121.38, 121.09, 117.14, 116.88; mp 179 °C.