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SYNTHESIS AND ANTITUMOR ACTIVITY OF BITHIENYL-PYRIMIDINE DERIVATIVES WITH ELECTROSTATIC BINDING SIDE CHAINS

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Abstract: A series of bithienyl-pyrimidines having cationic side chain have been developed as antitumor agents. This work illustrates the overwhelming importance of the bithienyl unit for efficient DNA binding. The X-ray structure of 4-(2',2"-thien-5-yl)-2-chloropyrimidine was obtained for postulating the conformation of the bithienyl-pyrimidine moiety.

DNA-interacting agents have been found to stimulate bleomycin-induced fragmentation of DNA.[1] Furthermore, flexible DNA binding agents are superior to the classical planar intercalators in catalyzing degradation of DNA by bleomycins. As a consequence, a great number of furan-, pyrrole- and thiophene-pyrimidine derivatives with cationic side chain have been synthesized as potential amplifiers for the bleomycins drugs.[2] Although these compounds possess binding affinity to DNA, they themselves lack *in vitro* antitumor activity. Our interest is to further develop the thiophene-pyrimidine series of unfused-polyaromatics into good antitumor agents. We felt that the most promising area for modification is the thiophene moiety. The goal is to replace this by the closely related bithiophene moiety while retaining the pyrimidine and cationic side chain. Bithienyls and terthienyl moieties are found in numerous natural products and possess important biological activity.[3] We report here the synthesis and antitumor activity of a series of novel 4-(2',2"-bithien-5'-yl)-2-substituted-pyrimidine analogues. The dimethylamino groups are used rather than the amidines as cationic side chain because they are more synthetically accessible.[4] We also synthesized the 4-thien-2-yl-pyrimidine derivatives for the purpose of comparing their antitumor activity.

The synthesis of 4-thien-2'-yl-2-substituted-pyrimidine and 4-(2',2"-bithien-5'-yl)-2-substituted-pyrimidine derivatives are shown in Scheme 1. The 4-thien-2'-yl-2-chloro-pyrimidine **1** and 4-(2',2"-bithien-5'-yl)-2-chloro-pyrimidine **2** were synthesized through the addition reaction of 2-lithiothiophene and 2-lithiobithiophene[5] with 2-chloropyrimidine, followed by aromatization of the dihydropyrimidine. The introduction of the cationic side chain can be readily accomplished by the nucleophilic displacement of the chloro-substitutent in **1** and **2**. Thus, the reaction of **1** and **2** with several dimethylamino alcohols and thiols gave the desired products **3a-c** and **4a-c**[6] in good yields.

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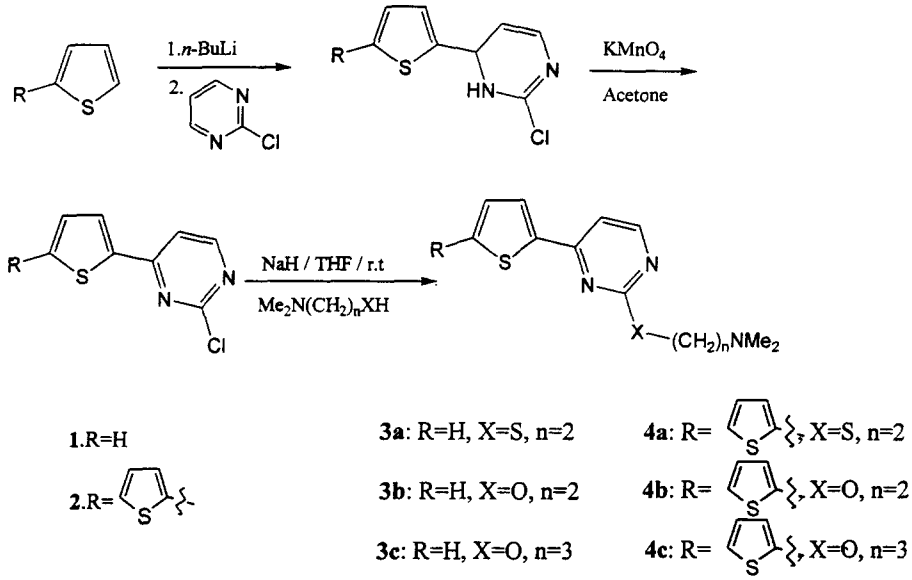

Scheme 1 Preparation of Thienyl-, Bithienyl-Pyrimidine Derivatives.

The mode of binding of this unfused polyaromatics is dependent on the conformation of the molecule. The 4-thien-2'yl-pyrimidine system has been reported to exist in solution in an essentially *s*-cis conformation.[7,8] No crystal structure has been reported. We were able to obtain the crystal structure for 4-(2',2"-bithien-5'-yl)-2-chloro-pyrimidine **2** as shown in Figure 1.[9] This showed the *s*-cis conformation between the thienyl and pyrimidine ring in the solid state, which is similar to that reported in solution. The bithienyl system is in a *s*-trans conformation. Assuming similar conformation for compound **4a-c**, this implied that the dimethylamino group lies in the concave face of the molecule, suitably orientated for electrostatic binding with the DNA phosphate groups.

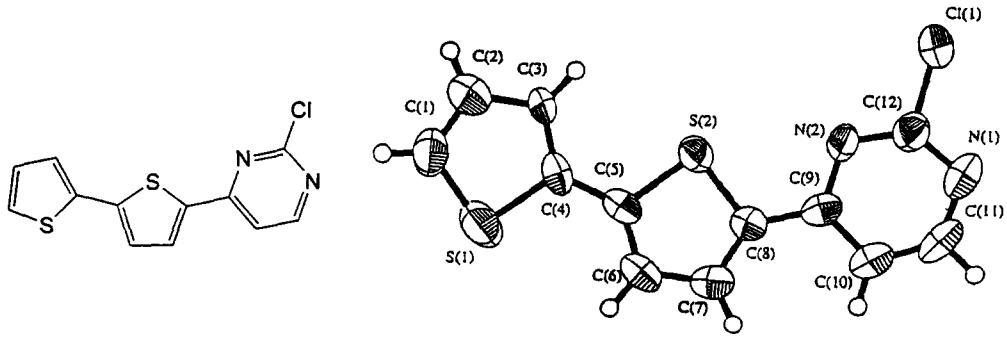

Figure 1.X-Ray Structure of 4-(2',2"-Bithien-5'-yl)-2-chloro-pyrimidine **2**.

The binding studies of **4a-c** were performed using fluorometric method at pH 8 as described by Morgan *et al.*[10] The binding constants, $K_{assoc.}$ /10^7 mol^{-1} for **4a-c** are of the following order: **4c**, 3.00; **4b**, 2.10 and **4a**, 0.65. In contrast, the binding affinities of **3a-c** were not measurable using this method (50% loss of the initial fluorescence cannot be achieved to enable the calculation of $K_{assoc.}$) , and previous report of similar analogues showed low binding constants.[2] The replacement of the thienyl unit in **3a-c** with a bithienyl unit in **4a-c** was found to drastically increase the binding affinity with DNA. This finding can be advantageously used in the design of new DNA binding antitumor agents.

Table 1. *In vitro* Cytotoxicity of Compounds **3a-c** and **4a-c**.

Compounds	Binding Constant PH=8	IC50 (μg / mL)			
		Hela	Hep-2	Colon-205	KB
3a	–[a]	>50[b]	>50	>50	>50
3b	–	>50	>50	>50	>50
3c	–	>50	>50	>50	>50
4a	$0.65x10^7$	25	≈50	30	≈50
4b	$2.10x10^7$	15	16	12	17
4c	$3.00x10^7$	7	11	7	5

[a] 50% loss of initial fluorescent cannot achieve.

[b] Drug concentration of 50μg / ml did not inhibit growth of cell by 50%

Cell cytotoxicity for compounds **3a-c** and **4a-c** were examined *in vitro* using four different cell lines, a human oral epidermoid carcinoma (KB), a human cervical carcinoma (Hela), a human larynx carcinoma (Hep-2) and human colon carcinoma (Colon-205) in a drug range of 1 - 50 μg/mL.[11] The results are summarized in Table 1 in term of IC_{50}. In the tested cell lines, thiophene-pyrimidine analogues **3a-c** were inactive (a dosage of 50 μg/mL does not cause 50% inhibition of cell growth). On the other hand, the bithiophene-pyrimidine congeners **4a-c** exhibit remarkably improved cytotoxicity. Thus the potent binding affinity of compound **4a-c** to DNA is required for cytotoxicity. Surprisingly the results reported here also clearly show dramatic effects of the heteroatom at the 2- position in the pyrimidine ring on cytotoxicity. The compound **4a** having a sulfur atom at the 2-position is less potent, with an IC_{50} in a drug range > 30 μg/mL. The compounds **4b,c** having an oxygen atom at the 2-position showed greatly improved cytotoxicity, with a drug range of 5 - 15 μg /mL.

In summary we have synthesized a series of bithienyl-pyrimidine analogues which showed remarkably improved cytotoxicity *in vitro* when compared to the thienyl-pyrimidine counterparts. There results illustrate the overwhelming importance of the bithienyl unit for efficient DNA binding which correlates well with the antitumor activity. Furthermore the crystal structure obtained for 4-(2',2"-bithien-5'-yl)-2-chloro-pyrimidine enable us to postulate the conformation of **4a-c** , crucial for the future understanding of the molecular basis for sequence selectivity.

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6. Compound **4a**: ^{1}H NMR ($CDCl_3$), orange oil, 8.47 (d, 1H), 7.83 (d, 1H), 7.52 (d, 1H), 7.50 (d, 1H), 7.42 (d, 1H) 7.38 (d, 1H), 7.21 (dd, 1H), 3.32 (t, 2H), 2.75 (t, 2H), 2.38 (s, 6H). Compound **4b**: mp 72-73°C. ^{1}H NMR ($CDCl_3$) 8.44 (d, 1H), 7.65 (d, 1H), 7.29 (d, 1H), 7.27 (d, 1H), 7.19 (d, 1H) 7.16 (d, 1H), 7.05 (dd, 1H), 4.54 (t, 2H), 2.79 (t, 2H), 2.36 (s, 6H). Compound **4c**: mp 67-68°C. ^{1}H NMR ($CDCl_3$) 8.44 (d, 1H), 7.66 (d, 1H), 7.30 (d, 1H), 7.27 (d, 1H), 7.20 (d, 1H) 7.16 (d, 1H), 7.06 (dd, 1H), 4.48 (t, 2H), 2.51 (t, 2H), 2.27 (s, 6H), 2.04 (m, 2H).
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