JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
*J. Phys. Org. Chem.* 2000; **13:** 344–346

# 2,6-Di(heteroarylvinyl)pyridines as new potential antitumor agents

**Maria Fichera, Luisa Gregoli and Giuseppe Musumarra***

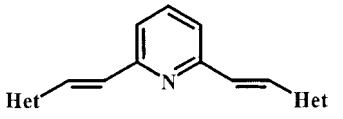
Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy

*Received 29 October; revised 28 January; accepted 28 February*

ABSTRACT: A circular dichroism spectroscopic study on the DNA binding ability of 2,6-di(heteroarylvinyl)pyridines (heteroaryl 2-furyl, 2-pyridyl, 2-thiazolyl) provided evidence for the binding of the furyl and thiazolyl derivatives to selected oligonucleotides. The furyl derivative, exhibiting $\log IC_{50} = -6.54$ for *in vitro* inhibition against breast carcinoma cells (MCF-7), might represent a new lead compound for structure optimization. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: 2,6-Di(heteroarylvinyl)pyridines; DNA binding; circular dichroism; *in vitro*; mammary carcinoma

Many antitumor drugs act by binding within the minor groove of double-helical DNA, interfering with both replication and transcription. The interest in the search for new structures able to bind selectively DNA minor groove GC bases, in which several oncogens are particularly rich, led to the recognition of the four Watson–Crick base pairs in the DNA minor groove by synthetic polyamides containing five-membered nitrogen heteroaromatics.[1] Many other DNA binding agents include aromatic or heteroaromatic moieties linked by carbon and/or heteroatom linkers, and amidino groups attached to the aromatic rings, including netropsin, distamycin, berenil and pentamidine, for which the crystal structure of the complex with a selected oligonucleotide dodecamer sequence is available.[2] Replacement of the central linkers in pentamidine and berenil with a furan moiety improves the DNA-binding properties, as confirmed by the crystal structures of the drug–DNA complexes.[3] Although the linker may not be directly involved in the DNA binding, its length is decisive for the binding selectivity. We have recently reported[4] on the synthesis and basic properties of the 2,6-di(heteroarylvinyl)pyridines **1**–**3** possessing structural skeletons in which heteroaromatic rings which may act as binders are linked by ethylenic double bonds exerting only a 'spacing' function. In this context, we now report a circular dichroism (CD) spectroscopic study on the DNA binding ability of 2,6-di(heteroarylvinyl)pyridines **1**–**3** and preliminary results on their *in vitro* antitumor activity towards two selected cell lines: human mammary carcinoma (MCF-7) and human non-small lung adenocarcinoma cells (A549).




| | Het | Code |
|---|---|---|
| 1 | furan-2-yl | $PF_2$ |
| 2 | thiazol-2-yl | $PTz_2$ |
| 3 | pyridin-2-yl | $P_3$ |

Compounds **1**–**3** have been used as ligands for double helices of the following dodecamer oligonucleotide sequences, selected as representative of AT-rich and GC-rich DNA fragments, respectively:

d[(ATAAATTAAATA) (TATTTAATTTAT)]
(AT rich)
d[(ATGGCCGCGCTA) (TACCGGCGCGAT)]
(GC rich)

CD spectra of **1**–**3** with AT- and GC-rich double helices did not show, apart from $PTz_2$ (see below), induced CD bands in the UV–visible region, where ligands exhibit absorption maxima, as is evident from those previously observed for mitomycin–DNA complexes.[5] CD measurements have been widely used to provide evidence of DNA conformational changes. As alteration of the DNA secondary structure or distorsion of the helix usually occurs upon binding of drug molecules, e.g. by intercalation, changes in the DNA CD bands were selected as a probe to detect conformational changes caused by the addition and consequent binding of **1**–**3** to the chosen helices. The study of the above changes is an area of great interest since drug-induced distorsions of

**Correspondence to:* G. Musumarra, Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy.
E-mail: gmusumarra@dipchi.unict.it
*Contract/grant sponsor:* University of Catania.
*Contract/grant sponsor:* MURST.
*Contract/grant sponsor:* CNR.

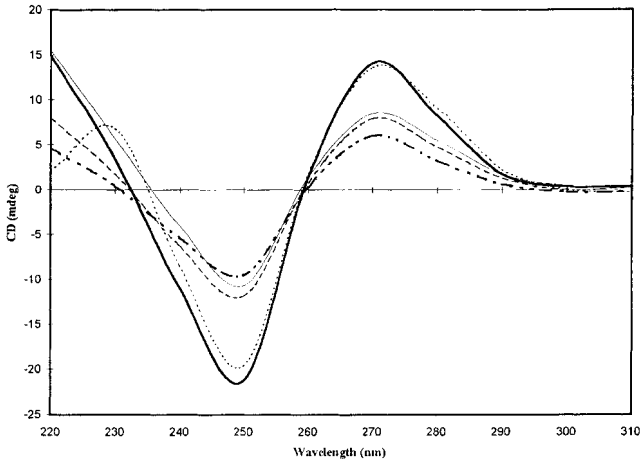



**Figure 1.** CD spectra at 25 °C (0.02 M Tris–HCl buffer, 0.1 M NaCl with 5% dioxane, pH 7.4) of $PF_2$ ligand and d[(ATAAATTAAATA) (TATTTAATTTAT)], at increasing ligand-to-DNA molar ratios: ·····, 0; -----, 0.1; —, 0.5; —, 1; -·-·-·-·-, 2

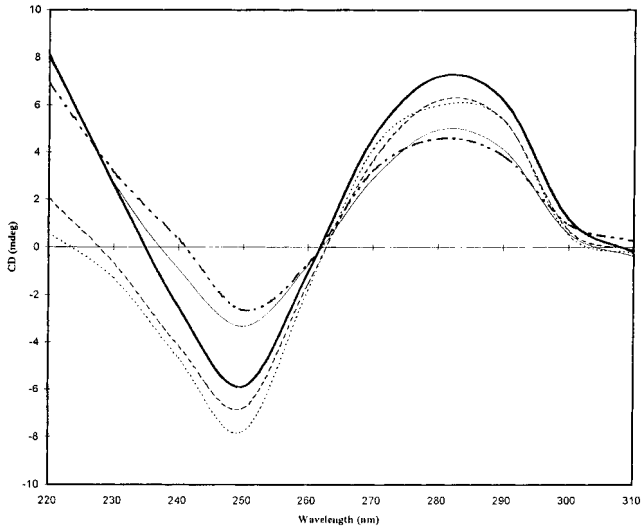



**Figure 2.** CD spectra at 25 °C (0.02 M Tris–HCl buffer, 0.1 M NaCl with 5% dioxane, pH 7.4) of $PF_2$ and d[(ATGGCCGCGCTA) (TACCGGCGCGAT)], at increasing ligand-to-DNA molar ratios: ·····, 0; -----, 0.1; —, 0.5; —, 1; -·-·-·-·-, 2

DNA structure play an important role in biological effects by interfering with repair, replication and transcription systems.

Figures 1 and 2 report the CD spectra of the furan derivative **1** at various stoichiometric ratios with respect to AT- and GC-rich helices. Upon ligand addition the intensities at the extrema of the dichroic bands at 248–270 and 250–284 nm, respectively, exhibit significant changes. At low ligand-to-DNA ratios the spectra show a hypochromic effect of the above bands, and similar smaller changes take place when small amounts of dioxane or ethanol are added to a solution of the selected DNA in buffer. This effect is well known and can be ascribed to a reduction of the base stacking forces such as van der Waals and solvation forces.[6] Therefore, the hypochromic effect observed in Figs 1 and 2 upon addition of 0.1 and 0.5 mol of ligand with respect to the double helix might be ascribed to an intercalation process that is always accompanied by unwinding of the helix and base unstacking. The spectra show a hyperchromic effect at equimolar ligand-to-DNA ratios and a subsequent hypochromic effect with an excess of ligand (2:1 ratio). This behaviour suggests that, when interacting with $PF_2$, both AT- and GC-rich helices undergo a sequence of DNA conformational changes probably due to helix stacking and unstacking processes involving the formation of different complexes in which one or more ligand molecules interact with the DNA double helix.

The CD spectra of the thiazole and pyridine derivatives **2** and **3** with the AT-rich helix exhibit only minor variations in the characteristic DNA range 210–300 nm up to a 2:1 molar ratio.

Figure 3 shows that, upon addition of one equivalent of $PTz_2$ to the AT- rich helix, a negative induced CD band appears in the characteristic ligand region above 300 nm. The above band, showing no significant variations upon addition of another equivalent of $PTz_2$ (2:1 ligand:oligo ratio) was not evident for the GC-rich helix. This finding suggests an interaction of $PTz_2$ with the AT rich helix. In conclusion, the present CD spectral investigation provides clear evidence for the interaction of $PF_2$ with both AT- and GC-rich helices and of $PTz_2$, with the AT-rich helix. Other interactions causing no significant CD changes, however, cannot be excluded.

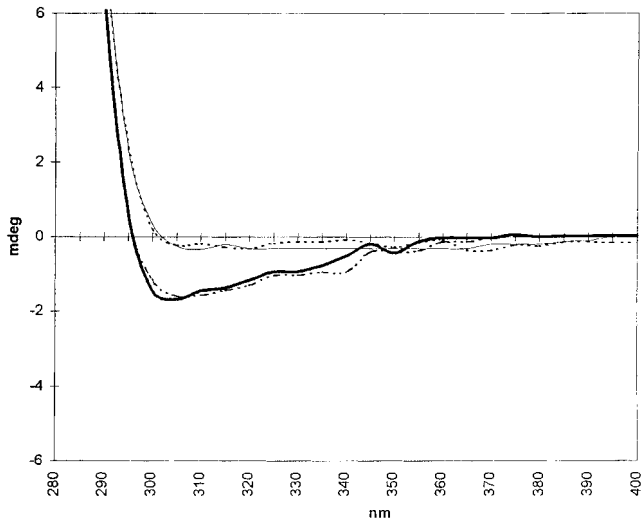



**Figure 3.** CD spectra in the range 280–400 nm at 25 °C (0.02 M Tris–HCl buffer, 0.1 M NaCl with 7.5% ethanol, pH 7.4) of $PTz_2$ and d[(ATAAATTAAATA) (TATTTAATTTAT)], at increasing ligand-to-DNA molar ratios: ·····, 0; —, 0.5; —, 1; -·-·-·-·-, 2

**Table 1.** Log$IC_{50}$ (μM)[a] for pyridines **1–3** and for other antitumor agents, with log$GI_{50}$ values from the NCI data bank (in italics) for comparison

| Cell line | *m*-AMSA NSC 249992 | Doxorubicin NSC 123127 | Tamoxifen NSC 180973 | $PF_2$ | $PTz_2$ | $P_3$ |
|---|---|---|---|---|---|---|
| MCF-7 | −7.1 | −9.0 | — | −6.54 (0.10) | −4.60 (0.06) | −5.01 (0.13) |
| | *−6.9*[b] | *−7.7*[b] | *−5.8*[b] | — | — | — |
| A549 | −7.2 | −8.0 | — | >−4 | −4.50 (0.02) | >−4 |
| A549/ATCC | *−7.3*[b] | *−7.1*[b] | *−5.2*[b] | — | — | — |

[a] Concentration to inhibit cell growth by 50%; 4-day drug exposure. Average values of two determinations; standard errors in parentheses.
[b] Log$GI_{50}$, 2 days drug exposure with correction for the cell count at time zero, from NCI data bank [database resources are currently available searching by chemical name or NSC number for a set of 175 standard agents in the World Wide Web (http://dtp.nci.nih.gov/docs)]

Table 1 reports the log$IC_{50}$ values for pyridines **1–3** and for *m*-AMSA and doxorubicin, antitumor agents with potentially similar mechanism. For example, *m*-AMSA acts against acute leukemia and malignant lymphomas by intercalation of the acridine chromophore and minor groove binding of the anisidine moiety.[7] Log$GI_{50}$ available in the NCI data bank for *m*-AMSA, doxorubicin and tamoxifen, a triarylethylene derivative exhibiting some structural analogy with **1–3**, are also reported for comparison. Compounds **1–3** were tested against two cell lines: breast carcinoma (MCF-7) and pulmonary adenocarcinoma (A549) human cells. All three compounds are more active against the breast cell line (MCF-7), with $PF_2$ being the most potent. This finding is consistent with the results of the CD study, pointing out major changes in the spectra of $PF_2$. Table 1 shows also that the activity of $PF_2$ against MCF-7 cells is comparable to that of *m*-AMSA, but lower than that of doxorubicin. The comparison with the log$GI_{50}$ from the NCI data bank confirms the above trend, but indicates that the activity of $PF_2$ against MCF-7 cells is comparable to or even better than that of the structural analogue tamoxifen. It is noteworthy that structures such as those of **1–3**, with three heteroaromatic rings spaced by ethylene linkers, have not yet been tested as antitumor agents. Such derivatives can be regarded as new 'lead compounds' for structure optimization. Studies aimed at the improvement of the solubility in water and hopefully of the antitumor activity are in progress.

## Acknowledgements

We thank Professor M. Stevens and Mr C. S. Matthews, Cancer Research Laboratories, University of Nottingham, UK, for the antitumor *in vitro* tests, and the University of Catania, MURST and CNR (Rome) for partial financial support.

## REFERENCES

1. (a) White S, Szewczyk JW, Turner JM, Baird EE, Dervan PB. *Nature (London)* 1998; **391**: 468; (b) Kielkopf CL, White S, Szewczyk JW, Turner JM, Baird EE, Dervan PB, Rees DC. *Science* 1998; **282**: 111.
2. Edwards KJ, Jenkins TC, Neidle S. *Biochemistry* 1992; **31**: 7104.
3. Trent JO, Clark GR, Kumar A, Wilson WD, Boykin DW, Hall JE, Tidwell RR, Blagburn BL, Neidle S. *J. Med. Chem.* 1996; **39**: 4554.
4. Ballistreri A, Gregoli L, Musumarra G, Spalletti A. *Tetrahedron* 1998; **54**: 9721.
5. Mercado CM, Tomasz M. *Biochemistry* 1977; **16**: 2040.
6. Wacker A, Lodemann E. *Angew. Chem., Int. Ed. Engl.* 1965; **4**: 150.
7. Hansch C, Sammes PG, Taylor JB. *Comp. Med. Chem.* 1990; **4**: 535.