Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 121–126

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS, DNA-DAMAGING AND CYTOTOXIC PROPERTIES OF NOVEL TOPOISOMERASE II-DIRECTED BISANTRENE ANALOGUES

Giuseppe Zagotto[1], Ambrogio Oliva[2], Fulvio Guano[3], Ernesto Menta[2], Giovanni Capranico[3] and Manlio Palumbo[1,*]

[1] Department of Pharmaceutical Sciences, University of Padova, Via Marzolo, 5, I-35131 Padova, Italy; [2] Research Center, Boehringer Mannheim Italia, Viale G.B. Stucchi, I-20052 Monza, Italy; [3] Istituto Nazionale Tumori, Divisione di Oncologia Sperimentale B, Via Venezian, 1, I-20133 Milano, Italy.

Received 14 May 1997; accepted 1 December 1997

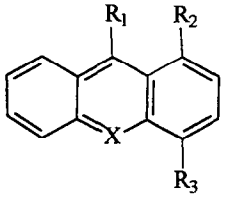
Abstract. New bisantrene analogues were synthesized, bearing one or two 4,5-dihydro-1H-imidazol-2-yl hydrazone side chains at positions 1,4 or 9 of the anthracene ring system. A 10-aza-bioisostere was also prepared. The position of substituents in structurally isomeric drugs modulates topoisomerase II poisoning and specificity, along with cytotoxicity.

Understanding the mechanism(s) by which anticancer drugs exert their action at the molecular level represents a major interest of the current medicinal chemistry. Indeed, many different targets have been identified and characterized, including DNA and enzymes involved in processing the nucleic acid. Among them, topoisomerase II poisons bear a special interest inasmuch as they stimulate DNA cleavage by forming a ternary complex, involving the enzyme, the drug and the nucleic acid [1]. Indeed, cleavable complex formation seems to represent a key step in eliciting cytotoxicity [2]. Interestingly, recognition of the macromolecular partners by the drug is not accidental, but exhibits well defined sequence specificity patterns along the DNA chain [3]. These are related to the nature and position of DNA-binding and enzyme-binding pharmacophoric groups present in the drug molecule. An interesting example is given by *m*-AMSA and bisantrene. They are chemically distinct, but share the same base preference for causing topoisomerase II-mediated DNA damage[4]. Theoretical studies carried out in our laboratories showed that these compounds exhibit a very satisfactory steric and electronic overlapping of stable conformations [4]. This suggests that they have common pharmacophoric features, and that the relative position of the planar portion and of the side-chain groups plays an important role in directing the topoisomerase poisoning effect and finally the cytotoxic activity. Incidentally, one of the bisantrene side-chains appears to be unnecessary for proper target recognition. To verify these ideas we have synthesized and investigated a number of anthracene derivatives having the following structural features (Figure 1): one 4,5-dihydro-1H-

Fax n° +39-49-8275711; E-mail mpalumbo@purple.dsfarm.unipd.it

0960-894X/98/$19.00
PII: S0960-894X(97)10207-4

imidazol-2-yl hydrazone side-chain group at position 9 (9-IHA) [5], the same substituent at position 1 (1-IHA), two 4,5-dihydro-1H-imidazol-2-yl hydrazone side-chains at positions 1 and 4 (1,4-IHA). The 10-aza derivative of 9-IHA (aza-9-IHA), comprising the side chain of bisantrene and the planar portion of *m*-AMSA, was also synthesized.



	R_1	R_2	R_3	X
bisantrene	a	H	H	C-a
9-IHA	a	H	H	CH
aza-9-IHA	a	H	H	N
1-IHA	H	a	H	CH
1,4-IHA	H	a	a	CH
m-AMSA	b	H	H	N

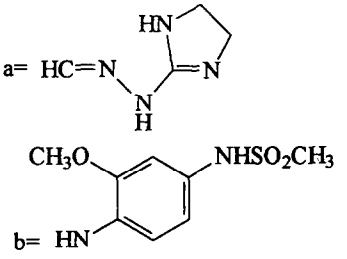


Figure 1. Chemical structure of the test drugs

Chemistry

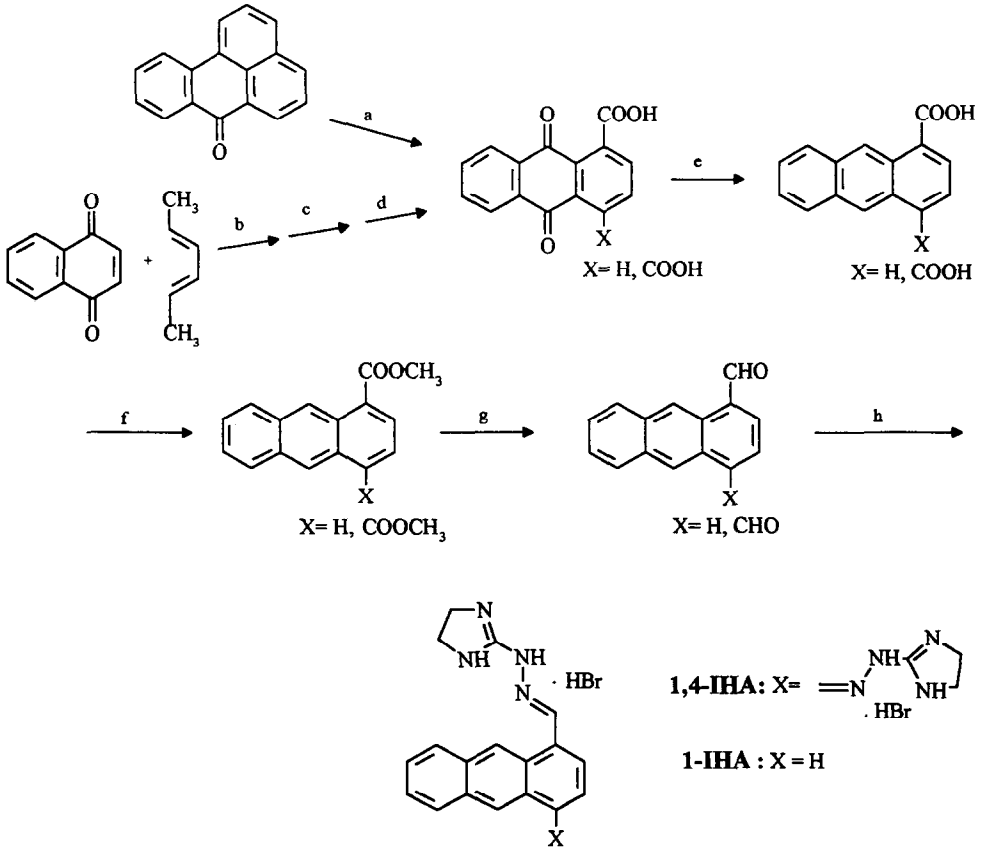
The general route to the desired compounds involved preparation of the appropriate carboxylic acid, followed by reduction to the aldehyde. The starting carboxylic acids were prepared according to known methods [6], but for the intermediate aldehydes an original route was followed. In fact, they were prepared by one step reduction of the carboxylic acid esters [7] using sodium bis (2-methoxy ethoxy) aluminum hydride deactivated with morpholine [8], whereas the literature reports use a two-step procedure involving the reduction of the anthracene carboxylic acid to the alcohol, followed by oxidation with pyridinium chlorochromate to the desired aldehyde [6]. The procedure described in this paper offers a number of advantages including the use of milder reagents, better yields and a one-step reduction. The 4,5-dihydro-1*H*-imidazol-2-yl-hydrazone side chain was finally introduced into the planar ring system by reaction of the carboxaldehyde with 2-hydrazino-2-imidazoline hydrobromide[3,9].

The synthetic steps to yield compounds 1-IHA and 1,4-IHA are reported in Scheme 1.

Topoisomerase II poisoning activity [10]

All new derivatives were tested for their ability to cause topoisomerase II-mediated DNA breaks. The level of DNA degradation observed for each compound in the presence of the enzyme is reported in Table 1. All test drugs, except 1,4-IHA, were able to produce topoisomerase-mediated cutting of DNA. This suggests that 9-IHA, aza-9-IHA and 1-IHA are able to interfere at the level of the topoisomerase II-nucleic acid cleavable complex. A preliminary analysis on sequencing gels using SV40 DNA fragments shows that the former two compounds exhibit, as theoretically proposed [4], cleavage patterns very similar to *m*-AMSA and bisantrene. On the contrary, 1-IHA exhibits a

substantially different intensity pattern, which indicates that this drug stimulates DNA damage at distinct sites along the genome (not shown).



a) CrO_3, HOAc, reflux, 66%; b) Toluene, 65 °C, 4 days, 87%;c) KOH/EtOH, 10 °C, 46%; d) HNO_3, reflux, 5 days, 31%; e) Zn/NH_4OH, 80 °C, 49%; f) Me_2SO_4, K_2CO_3, 2-butanone, reflux, 74-81%; g) RedAl/morpholine, toluene-15°C, 71-79%; h) 2-hydrazino-2-imidazoline·HBr, EtOH, reflux, 60-71%.

Scheme 1

Cell cytotoxicity [11]

Cell cytotoxicity was examined in vitro using two different cell lines, a human ovarian cancer (A2780) and a human promyelocytic leukemia (HL60) in the drug range 0.1-100 μM. The results are summarized in Table 1 in terms of IC_{50}. In the tested lines *m*-AMSA and bisantrene behave

similarly, as both exhibit very close IC_{50} values in the sub-micromolar range. All IHA derivatives, although cytotoxic, are less potent. Surprisingly, 1,4-IHA, which is not able to poison topoisomerase II, proves to be the most effective. As expected, the mono-substituted congeners exhibit a remarkably reduced affinity for DNA when compared to bisantrene (data not shown). This might account for a less efficient poisoning of the cleavable complex and a lower cytotoxic action.

Table 1. Topoisomerase II-mediated DNA damage and cytotoxicity of IHA compounds

Compound	Percent uncut DNA[a]			IC_{50} (μM)[c]	
	0.1[b]	*1*[b]	*10*[b]	*A2780*	*HL60*
m-AMSA	12	4	1	0.35±0.03	0.40±0.07
Bisantrene	14	46	57	0.34±0.02	0.30±0.05
9-IHA	41	30	11	37.8±7.2	73.5±10.3
aza-9-IHA	51	38	25	28.1±1.3	48.1±9.4
1-IHA	56	48	20	18.6 ±4.7	23.0±3.1
1,4-bis-IHA	54	103	95	4.8± 1.9	11.2±5.4

[a] Percent of DNA remaining uncut after incubation with the enzyme at the indicated drug concentration. Topoisomerase II produces about 50% cutting in the absence of drugs.
[b] Drug concentration (μM).
[c] IC_{50} values are the average of two independent experiments ± SE.

The results reported here clearly show dramatic effects of the substitution pattern in the bisantrene family. All compounds, except 1,4-IHA, are topoisomerase II poisons. This confirms, as theoretically inferred [4], that one side-chain in bisantrene is sufficient to grant interference with the enzyme and, moreover, that appropriate linking of residues from different drugs may indeed give effective compounds. The level of cleavage observed depends on the nature of each derivative, which, on turn, is related to the combination of poisoning and inhibition effects, generated by blocking enzyme cycle progression or by competing with topoisomerase for binding to the DNA template. The latter effect is evident for the bis-substituted drugs, exhibiting prominent affinity for DNA.

Maintaining the side chain groups at position 9 of the anthracene ring system produces a sequence pattern of topoisomerase II poisoning by 9-IHA and aza-9-IHA very similar to the parent *m*-AMSA and bisantrene. This represents an additional indication that this class of compounds shares a common pharmacophore indeed. On the other hand, the isomeric derivative 1-IHA is also able to stimulate topoisomerase II-mediated DNA damage to a noticeable extent, but it exhibits a clearly

distinct cleavage pattern, which points to different pharmacophoric interactions. That the relative position of the planar structure and side-chains plays a fundamental role in stabilizing the cleavable complex through specific contacts with the enzyme and a given DNA sequence is further strengthened by the suppression of cleavage stimulation when using the bisantrene isomer 1,4-IHA (Table 1).

The cytotoxic effects of all new compounds are remarkably reduced when compared to *m*-AMSA and bisantrene. The levels of DNA damage for the test drugs (except for 1,4-IHA) compare reasonably well with the cell killing effects shown by the same compounds. In fact, the most cytotoxic *m*-AMSA and bisantrene show substantial DNA cutting at the lowest concentrations tested, while comparable effects can be seen for the congeners only at 10 μM or higher. Further modulation of cytotoxicity should be ascribed to the pharmacokinetic properties of individual drugs. Interestingly (and unexpectedly) 1,4-IHA is the most cytotoxic among the new derivatives, although it suppresses, instead of stimulating, topoisomerase II-mediated DNA damage (Table 1). Hence, this structural isomer of bisantrene is able to induce cell death through a mechanism of action distinct from that displayed by bisantrene itself. Therefore, 1,4-IHA may represent an interesting lead for developing effective anticancer drugs with alternative activity patterns.

References and Notes

1. a) Liu, L.F. *Annu. Rev. Biochem.* **1989**, *58*, 351-375; b) Capranico, G.; Binaschi, M.; Borgnetto, M. E.; Zunino, F.; Palumbo, M. *Trends Pharm. Sci.* **1997**, *18*, 323-329.
2. Pommier, Y.; Capranico G.; Orr, A.; Kohn, K.W. *J. Mol. Biol.* **1991**, *222*, 909-924.
3. a) Capranico, G.; Palumbo, M.; Tinelli, S.; Zunino, F. *J. Biol. Chem.* **1994**, *269*, 25004-25009; b) De Isabella, P.; Zunino, F.; Capranico, G. *Nucleic Acids Res.* **1995**, *23*, 223-229; c) Capranico, G.; Butelli, E.; Zunino, F. *Cancer Res.* **1995**, *55*, 312-317.
4. Capranico, G.; Palumbo, M.; Tinelli, S.; Mabilia, M.; Pozzan, A.; Zunino, F. *J. Mol. Biol.* **1994**, *235*, 1218-1230.
5. Murdock, C.; Child, R. G.; Yang-i, L.; Warren, J. D.; Fabio, P. F.; Lee Ving, J.; Izzo, P. T.; Lang, S. A.; Angier, R. B.; Citarella, R. V.; Wallence, R. E.; Durr, F. E. *J. Med. Chem.* **1982**, *25*, 505-518.
6. a) Garay, R.O.; Naarmann, H; Mullen, K. *Macromolecules* **1994**, 27, 1922-1927. b) Bair, K.W.; Andrews C.W.; Tuttle, R.L.; Knick, V.C.; Cory, M; McKee, D. *J. Med. Chem.* **1991**, 34, 1983-1990; c) Muth, C. W.; Minigh, H. L.; Chookruvong, S.; Hall, L. A.; Chao, H. -T. *J. Med. Chem.* **1981**, 24, 1016-1018.

7. *Anthracene-1-carboxylic acid methyl ester.* ^{1}H NMR ($CDCl_3$): δ 9.58 (s, 1 H); 8.47 (s, 1 H); 8.25 (dd, 1 H, $J_{1,3}$=7.1 Hz, $J_{1,4}$= 1.2 Hz); 8.19 (dd, 1 H; $J_{1,3}$=8.8 Hz, $J_{1,4}$=1.1 Hz); 8.12-7.98 (m, 2 H); 7.54-7.44 (m, 3H); 4.06 (s, 3H); *Anthracene-1,4-dicarboxylic acid methyl ester.* ^{1}H NMR ($CDCl_3$): δ 9.42 (s, 2H); 8.06 (s, 2H); 8.01 (dd, 2H, $J_{1,3}$=6.4 Hz, $J_{1,4}$=3.3 Hz); 7.47-7.20 (m, 2H); 4.01 (s, 6H); *9-Acridinecarboxylic acid, methyl ester.* ^{1}H NMR ($CDCl_3$): δ 4.22 (s, 3H); 7.56-7.78-7.89 (m, 2H); 7.82 (m, 2H); 8.03 (d, 2H); 8.28 (d, 2H).

8. Kanazawa, R.; Tokoroyama T. *Synthesis* **1976**, 526-527.

9. *Anthracene-1,4-dicarboxaldehyde-(4,5-dihydro-1H-imidazol-2-yl)-dihydrazone hydrobromide* (1,4-IHA). To anthracene-1,4-dicarboxaldehyde (0.096 g, 0.41 mmol) dissolved in 25 ml of ethanol 2-hydrazino-1-imidazoline hydrobromide (0.191 g, 1.06 mmol) was added. The reaction mixture was refluxed for 36 hours and then the solvent was evaporated. The residue was crystallized from absolute ethanol to yield 0.163 g (71%) of pure product as dihydrobromide. mp 325 (decomposes). ^{1}H NMR (DMSO-d_6): δ 12.48 (s, 2H); 9.27 (s, 2H); 9.14 (s, 2H); 8.88 (s br., 4H); 8.26 (s, 2H); 8.28-8.14 (m, 2H); 7.70-7.62 (m, 2H); 3.93 (s, 8H). ^{13}C-NMR ($CDCl_3$): δ 43, 123, 126, 127, 128, 129, 131, 132, 147, 158. IR (KBr 1%) cm^{-1}: 3217,1655, 1611, 1480, 755. *Anthracene-1-carboxaldehyde-(4,5-dihydro-1H-imidazol-2-yl)-hydrazone hydrobromide* (1-IHA). mp 235 (decomposes). ^{1}H NMR ($CDCl_3$): δ 12.35 (s br., 1 H); 9.08 (s, 1H); 8.89 (s, 1 H); 8.39 (s, 1H); 8.19 (br. s, 2H); 8.27-7.76 (m, 4H); 7.47-7.35 (m, 3H); 3.77 (s, 4H). ^{13}C-NMR ($CDCl_3$): δ 124, 125, 127, 127, 128, 129, 129, 129, 129, 130, 132, 132.1, 132.6, 132.8, 150, 159. IR (KBr 1%) cm^{-1}: 3250, 1661, 1413, 753. *9-Acridinecarboxaldehyde (4,5-dihydro-1H-imidazol-2-yl)-hydrazone hydrobromide* (Aza-9-IHA) mp 245-246 °C. ^{1}H NMR (DMSO-d_6): d 3.78 (s, 4H); 7.67-7.77 (m, 2H); 7.87-7.97 (m, 2H); 8.23 (d, J = 9.1 Hz, 2H); 8.52 (d, J = 8.3 Hz, 2H); 8.85 (br. s, 2H, exchangeable with D_2O); 9.38 (s, 1H); 12.65 (br. s, 1H, exchangeable with D_2O).

10. DNA cleavage levels were determined by Phosphorimager analysis of agarose gels using 5'-end ^{32}P-labelled SV40 DNA fragments in the presence of the test compounds at 0.1, 1 and 10 μM concentration.

11. A2780 and HL60 cancer cell lines were cultured in RPMI 1640 medium plus 10% fetal calf serum. Drug treatments were for 1 hr at 37°C, using exponentially growing cells. After drug treatment, cells were washed twice, resuspended in drug-free medium, cultured for 72 hr and then counted, to evaluate the percentage of survival in comparison to untreated cells.