SYNTHESIS, TRYPANOCIDAL ACTIVITY AND DNA BINDING OF NEW BENZO[b][1,8]-NAPHTHYRIDONES DERIVATIVES

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ABSTRACT

A novel set of N-alkyl naphthyridone compounds has been prepared and characterized. These compounds have been compared to analogous 9-acridinones for a possible trypanocidal activity. With reference to this, naphthyridones seem to be more promising compounds than the 9-acridinone ones.

INTRODUCTION

Within the scope of the search of new trypanocidal agents (1-3) we have recently prepared and tested some 1,4-dimethoxy-9-(10H)-acridinone derivatives (4). As the naphtyridine nucleus is structurally related to the acridine one, we were also interested in preparing and investigating antiparasitic activity of some new benzo [b][1,8]-5-naphthyridone derivatives, substituted similarly as acridinones already tested.

EXPERIMENTAL

1 - Chemistry

Melting points were determined in open capillary tubes on a Buchi-Tottoli apparatus and are given uncorrected. NMR spectra were recorded at 20.00 ± 0.1°C, using a Bruker AM 200 spectrometer. Microanalyses were carried out using a Technicon CHN autoanalyzer.

Général procedure for synthesis of compounds 1-8 (scheme 1)

Procedure for compounds 1 and 2

A mixture of freshly distilled aniline or properly substituted anilines (80 mmol), 2-chloro nicotonic acid (80 mmol), copper (0.8 g) and xylene (200 ml) is refluxed for 24 h with stirring. After filtration, the mixture is allowed to cool to room temperature. The precipitate obtained is recrystallized.

Procedure for compounds 3 and 4

Anthranilic acids 1 or 2 (10 mmol) are refluxed for 4 h with polyphosphoric acid in excess (more than 20 fold the weight of anthranilic acid). Then, the mixture is poured on ice and ammonia is slowly added. The precipitate obtained is recrystallized.

Procedure for compounds 5 and 6

A mixture of naphthyridone 3 or 4 (10 mmol), alkylating agent (15 mmol), and triethylbenzylammonium chloride hydrochloride (5 mmol), aqueous 50 % potassium hydroxide (50 ml), and toluene (100 ml) is refluxed for 24 h with stirring. Then, the organic layer is separated, washed with water, and dried with anhydrous sodium sulfate. After evaporation of solvent, crude product is recrystallized.

Procedure for compound 7

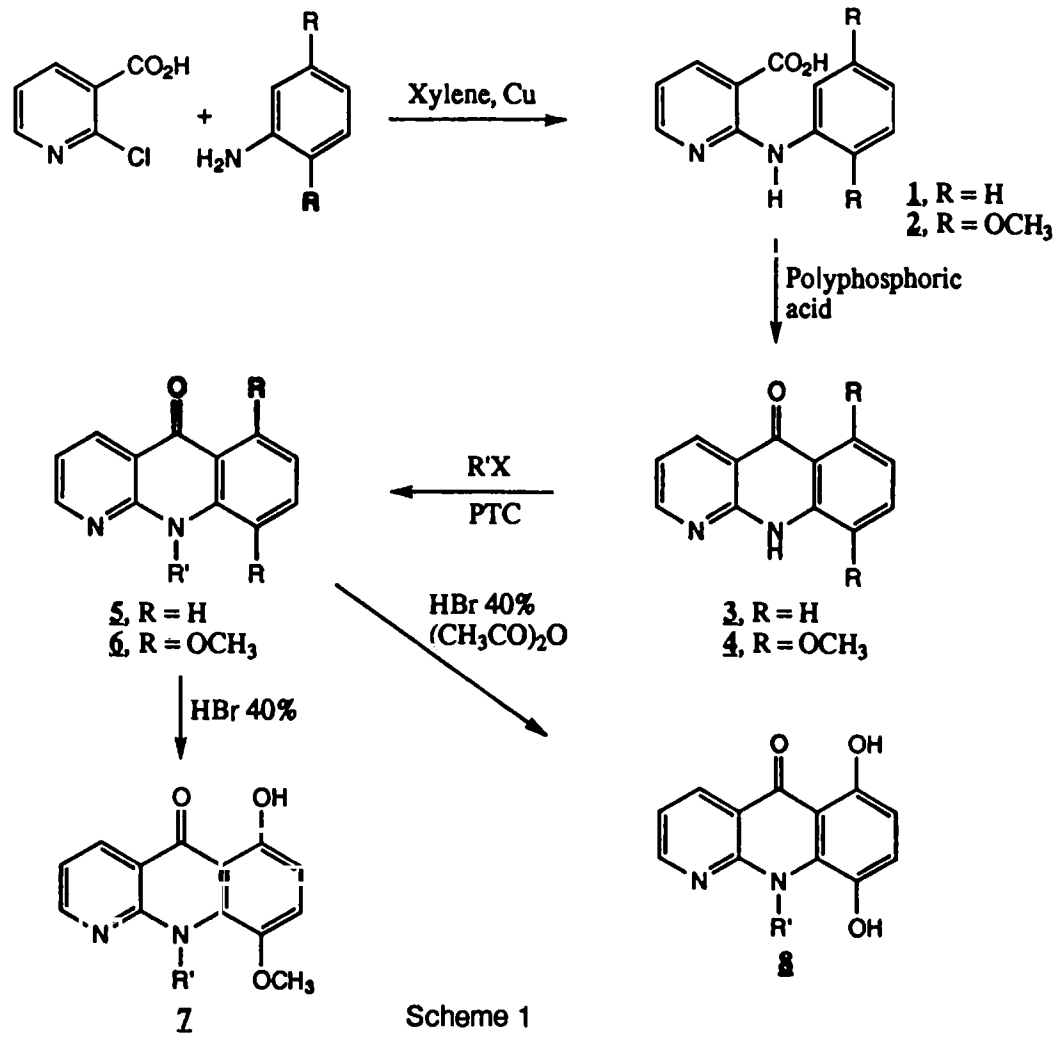
A mixture of 6 (2 mmol) and 40 % hydrobromic acid (320 mmol), is refluxed for 4 h with stirring before being allowed to cool to room temperature. Solution is then neutralized with ammonia, with cooling to maintain the temperature at 0° C. The precipitate obtained is recrystallized.

Procedure for compound 8

A mixture of 6 (2 mmol), acetic anhydride (34 mmol), and 40 % hydrobromic acid (145 mmol) is refluxed for 3 days with stirring before to be left in a fridge for a night. The precipitate obtained is washed with water before to be recrystallized.

2 - Parasitology

Drugs were tested during the exponential growth-phase of parasites. The strain of *Trypanosoma cruzi* was isolated from a clinical case (Institute of Malariology in Macaray, Venezuela). Epimastigote forms of parasites were cultured at 28° C in Liquid Trypanosoma medium (5). Assays were performed in multiwell plates.



Scheme 1

The number of parasites was $5x10^5$ parasites/ml. After treatment with compounds, plates were centrifuged at 2000 g and washed 3 times with PBS-0.05 % Tween 20. Parasites were then fixed with methanol and stained with 1 % eosin in water during 30 min at 37 ° C. The stain solution was removed and washed 3 times with tap water. The number of parasites was estimated using a Kontron ST 210 analyzer according to the procedure of Finlay et al (6).

3 - Binding to DNA

The binding of drugs to calf thymus DNA was spectrophotometrically measured. Spectrophotometric measurements were carried out in 0.018 mol/l NaCl - 0.03 mol/l Tris Cl Buffer (pH = 7.0), using a Pye-Unicam SP 8000 spectrophotometer fitted with a Pye-Unicam SP 876 series 2 temperature programme controller. Conditions were those previously described (7). Binding constants were calculated using "Enzfitter", a non linear regression analysis programme from Elsevier Biosoft.

4 - Acute toxicity

Acute toxicity was estimated in 20 g weight male IOPS mice, according to the Lorke (8) procedure. Compounds either were dissolved in saline or were suspended in olive oil (pharmaceutical grade). Aliquots of dilutions were intraperitoneally delivered.

5 - Conformational analysis

Strain energies have been calculated using the Molecular Mechanics Programme MAXIMIN II from the SYBYL software (Tripos Inc.). Graphics were displayed on ESV-10/32 workstation (Evans and Sutherland).

RESULTS AND DISCUSSION

Synthetic pathways are summarized in Scheme 1.

The benzo [b][1,8]-5-naphthyridone,3, was obtained from aniline and 2-chloronicotinic

acid dissolved in xylene, while the 6,9-dimethoxy derivative, 4, was obtained from 1,5-dimethoxyaniline according to the same procedure.

Compounds 3 and 4 were alkylated under phase transfer catalysis conditions, leading so to the 10-alkyl derivatives 5 and 6.

The 6-hydroxy-9-methoxy-10-alkyl-benzo[(b][1,8]-5-naphthyridones, 7, were then prepared demethylating 6 with hydrobromic acid whilst demethylation of the same, achieved with a mixture of hydrobromic acid and acetic anhydride, led to 6,9-dihydroxy-10-alkyl-benzo[(b][1,8]-5-naphthyridones, 8. However compounds 7 and 8 cannot be obtained directly alkylating hydroxy-methoxy-naphthyridone or dihydroxy-naphthyridone.

Chemical data about compounds prepared, are collected in Table 1.

Furthermore, compounds 5c, 6c, 7b and 8b were tested against epimastigote forms of *Trypanosoma cruzi*.

Moreover, acute toxicity of drugs when delivered intraperitoneally, was evaluated in mice. Results are given in Table 2, comparatively to those from 10-(2'-diethylaminoethyl)-9-acridinone, 9, 1,4-dimethoxy-10-(2'-diethylaminoethyl)-9-acridinone, 10, 1-hydroxy-4-methoxy-10- (2' diethylaminoethyl)-9-acridinone, 12, and from 1,4-dihydroxy-10-(2'-diethylaminoethyl)-9-acridinone, 11.

One must noted that activity is significantly higher in the naphthyridone series than in the acridinone one, while toxicity is about the same order of magnitude, on the condition that the same solvent is used.

In contrast, DNA binding data (Table 3) are closely related in both series. This is presumably due to the presence in each case of a basic side chain which is protonated at pH 7.0.

Thus, intercalation into DNA would be stabilized by anionic interaction between extracyclic protonated nitrogen and anionic phosphate backbone of the nucleic acid. However, there is a lack of any correlation between trypanocidal activity and DNA intercalation, as

Table 1 : Chemical data

Compound *	R	R'	Mp (°C)	Yield (%)	^{1}HNMR d (ppm) and multiplicity **	Solvent ***
1	H	-	152	53	9.1 (d,1H) ; 8.1 (d,1H) ; 7.7 (d,3H) ; 7.5 (t 2H) ; 7.2 (t, 2H)	TFA-d
2	OCH_3	-	120	63	10.9 (s,1H) ; 8.4 (m,2H) ; 8.2 (dd,1H) ; 7.0 (m, 2H) ; 6.4 (dd,1H) 3.8 (s, 3 H) 3.6 (s, 3H)	DMSO-d_6
3	H	-	279	96	12.2 (s, 1H) ; 8.6 (dd 1H) ; 8.55 (dd, 1H) ; 8.2 (dd, 1H) ; 7.7 (m 2H) ; 7.3 (m 2H)	DMSO-d_6
5a	H	CH_3	248	80	8.8 (m 2 H) ; 8.5 (dd,1H) ; 7.85 (dd,1H) ; 7.65 (dd 1H) ; 7.3 (m, 2H) ; 4.15 (s, 3 H)	CDC_3
5b	H	C_2H_5	150	74	8.8 (m 2 H) ; 7.6 (dd, 1H) ; 8.55 (dd, 1H) ; 7.85 (td, 1H) ; 7.3 (m, 2H) ; 4.85 (q, 2H) ; 1.5 (t, 3H)	CDC_3
5c	H	$(CH_2)_2$-$N(C_2H_5)_2$	130	60	9.5 (d,1H) ; 9.3 (d,1 H) ; 8.8 (d 1H) ; 8.2 (t, 1H) ; 7.8 (m 3 H) ; 5.4 (t, 2H) ; 4.0 (t, 2 H) ; 3.6 (q 4H) ; 1.6 (t, 6H)	TFA - d
4	OCH_3	-	188	95	9.25 (s, 1H) ; 8.7 (m, 2H) ; 7.3 (m,1H) ; 7.1 (d 1H) ; 6.6 (d,1H) ; 4.0 (s, 6 H)	CDC_3
6a	OCH_3	CH_3	240	40	9.7 (d,1 H) ; 9.0 (d 1 H) ; 7.9 (t,1H) ; 7.7 (d 1H) ; 7.2 (d,1H) ; 4.7 (s, 3 H) ; 4.2 (s, 3 H) ; 4.2 (s 3 H)	TFA - d
6b	OCH_3	C_2H_5	110	18	8.7 (dd, 2 H) ; 7.3 (m, 2H) ; 6.8 (d, 1H) ; 4.8 (q, 2 H) ; 3.95 (s, 3 H) ; 4.1 (s, 3 H) ; 1.35 (t, 3H)	CDC_3
6c	OCH_3	$(CH_2)_2$-$N(C_2H_5)_2$	95	60	8.7 (m, 2 H) ; 7.2 (m 2 H) ; 6.7 (d 1 H) ; 5.0 (s, 2H) ; 4.15 (s, 3 H) ; 4.0 (s, 3 H) ; 3.0 (t, 2 H) ; 2.6 (q, 4 H) ; 1.0 (t, 6 H)	CDC_3
7a	-	H	200	84	12.4 (s, 1H) ; 9.4 (s, 1H) ; 8.8 (m 2H) ; 7.3 (m, 1H) ; 7.2 (d, 1H) ; 6.6 (d, 1 H) ; 4.0 (s,3 H)	CDC_3
7b	-	$(CH_2)_2$-$N(C_2H_5)_2$	256	62	10.3 (s, 1H) ; 8.8 (dd, 1H) ; 8.6 (dd, 1H) ; 7.3 (m, 2 H) ; 6.7 (d, 1 H) ; 5.2 (t, 2 H) ; 4.0 (s, 3H) ; 3.8 (t, 2H) ; 3.4 (q, 4H) ; 1.6 (t, 6H)	CDC_3
8a	-	H	299	67	12.7 (s,1H) ; 11.2 (s,1H) ; 9.9 (s,1H) ; 8.9 (dd, 1H) ; 8.6 (dd, 1H) ; 7.4 (m, 1H) ; 7.2 (d, 1H) ; 6.5 (d, 1H)	DMSO-d_6
8b	-	$(CH_2)_2$-$N(C_2H_5)_2$	270	72	13.0 (s, 1H) ; 10.2 (s, 1H) ; 9.7 (s 1H) ; 9.0 (t, 1H) ; 8.7 (dd, 1H) ; 7.6 (m, 2H) ; 6.8 (d, 1H) ; 5.2 (t, 2H) ; 4.0 (t, 2H) ; 3.4 (q, 4H) ; 1.4 (t, 6H)	DMSO-d_6

* Analyses agree within 0,4 % of the theoretical values
** Abreviations have their usual significance
*** With TMS as internal standard

Table 2 - Acute toxicity in mice and growth inhibitory effect on *T.cruzi* strains at 24 h, 48 h and 72 h time of incubation ,with drug concentrations of 100 µg/ml, 10 µg/ml, and 1 µg/ml.

LD 50 i.p. (mg/kg)		Trypanocidal Activity								
		Time of incubation (h)	24			48			72	
W*	O*	Cpd \ Drug concn µg/ml	100	10	1	100	10	1	100	10	1
100		5c	90	70	60	100	85	80	100	90	90
120		6c	100	100	85	100	100	90	100	100	100
190	380	7b	90	90	75	100	100	100	100	100	100
200	400	8b	100	95	90	100	100	95	100	100	100
	200	9	100	50	30	100	90	55	100	100	80
	250	10	00	0	0	70	0	0	100	0	0
	380	11	100	45	0	100	80	0	100	95	0
	400	12	100	0	0	100	30	0	100	30	0
		nifurtimox **	100	60	55	100	100	65	100	100	75

* W = Water ; O = olive oil
* * drug used as reference

Table 3 - Binding data for interaction of selected test-sample compounds with DNA

Compound	Bathochromic shift (nm)	Affinity constant ($mol^{-1} \times 10^6$)	N° of binding sites/ moles DNA
3	-	-	-
5b	-	-	-
5c	2	0.35	0.19
6c	10	1.54	0.32
7b	5	1.45	0.22
9	2	0,84	0,20
10	10	1,97	0,20
11	10	1.51	0.20
12	5	4,08	0,20

Table 4 - Strain energies and values of the dihedral angle α for the acridinone 13, and for the naphthyridone 14.

α (°)	Energy (kcal / mol)	
	13	14
167	4.27	3.25
164	3.57	2.87
159	2.98	2.75
152	**2.74**	2.93
147	4.20	4.24

already observed (4). Hence, the primary biological target for the drugs investigated has still to be portrayed.

Now, there are no great differences between acridinones and naphthyridones, as regards the geometrical structure of heterocyclic nucleus. This was demonstrated by conformational analysis of 10-methyl-9-acridinone,13 and 10-methyl-5-naphthyridone, 14. Results are collected in Table 4.

Indeed, the most probable value of the dihedral angle α between the side aromatic rings

of the heterocycle, when the molecule is folded along an hypothetical axis N (10)-0, approximates 150° for the acridinone derivative and 160° for the naphthyridone one. Hence, molecular structures are quite similar, so we can conclude that differences in the activity under investigation, do not depend upon geometrical features.

In conclusion, substitution of a benzo ring in the acridine nucleus with a pyridine ring, suitably optimizes the tricyclic support of trypanocidal activity.

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