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Original article

Synthesis and antiproliferative activity of oxazinocarbazole and *N*,*N*-bis (carbazolylmethyl)amine derivatives

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ABSTRACT

The synthesis, structure elucidation and antitumoral activity of novel heterocyclic compounds containing a carbazole nucleus are reported. Oxazinocarbazoles were synthesized by application of the Mannich reaction to the corresponding hydroxylated derivatives leading to 41 new molecules. Their cytotoxic activity was evaluated against various human tumor cell lines including three leukemic cell lines: CEM and Jurkat (type T), Raji (type B); breast cancer cell line (MCF-7); colorectal cancer cell line (Caco-2).

A primary screening at 100 μM allowed the selection of the 10 most active compounds, which showed an antiproliferative activity on all the cell lines. A dose–effect study between 12.5 and 100 μM sorted two compounds with a significant activity: **5t** and **7e** against leukemic cell lines CEM, Jurkat and Raji with IC_{50} values around 12 μM.

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1. Introduction

Many condensed heterocyclic compounds containing a carbazole nucleus have been reported to develop a broad range of potent biological activities, notably anticancer activity [1]. Among these, natural or synthetic pyridocarbazoles [2,3], indolocarbazoles [4–8], pyranocarbazoles [9,10], pyrrolocarbazoles [11,12], benzocarbazoles [13] or simply tricyclic carbazoles [14–17] have been reported. On the other hand, oxazine derivatives have also shown a wide range of biological activities, such as anti-inflammatory [18] and antitumoral properties [19–21].

In the course of our studies on the chemistry and pharmacology of carbazole derivatives [22–24], we prepared various oxazinocarbazoles as cytotoxic agents. Here we describe the synthesis of some oxazino[5,6-*a*]-, -[5,6-*c*]- and [6,5-*b*]carbazoles through a Mannich type reaction starting from 2- or 4-hydroxycarbazoles, and their antiproliferative activity towards five human tumor cell lines: CEM (a T cell leukemia cell line), Raji (Burkitt's lymphoma), Jurkat (an acute T cell leukemia), MCF-7 (breast cancer cells) and Caco-2 (colorectal cancer). The cytotoxic activity of some by-products obtained by a dimerization process in the course of the Mannich condensations was also evaluated.

2. Chemistry

We first started with hydroxycarbazoles **1** and **2** but the kinetics of their aminomethylations where slow and a large amount of degradation products was formed. These observations led us to selectively synthesize the *N*-alkylated hydroxycarbazoles **3** and **4**. The protection of the indolic nitrogen with an alkyl group was expected to give more stability and better reactivity to hydroxycarbazoles in the Mannich reaction.

The synthesis of *N*-substituted 2- and 4-hydroxycarbazoles is outlined in Scheme 1.

Compounds **3a** [25] and **3b** [26] were already described. They were obtained by reducing the corresponding tetrahydrocarbazol-4-ones. Compound **4b** was obtained by Muth in 1935 [27] by *N*-alkylation of 2-ethoxycarbazole and subsequent deprotection of the hydroxy group. Compound **4d** was obtained by Joshi et al [28] by reaction of 2-hydroxycarbazole with 2-methylbut-3-en-2-ol. On the other hand, it is known that hydroxycarbazoles are easily *O*-mono alkylated in the presence of one equivalent of an alkylating agent RX and of a base like *n*-BuLi or NaH in anhydrous conditions. The *N*,*O*-bis

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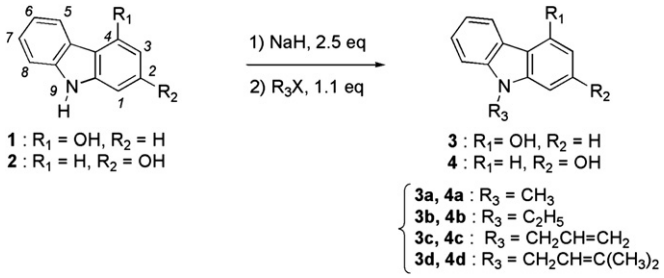


Scheme 1. Synthesis of N-substituted hydroxycarbazoles **3** and **4**.

alkylations are obtained by using an excess of the base and of RX. Albanese et al [29] described in 1995 the chemoselective *N*-alkylation of some 2-hydroxycarbazoles, which was achieved by generating the *N*,*O*-dianion with 2.5 equivalents of NaH in DMF/THF mixture under argon atmosphere at room temperature and subsequent treatment with the alkylating agent. The reaction rate and selectivity are strongly affected by the reaction conditions: solvent, temperature, base and leaving group X of the alkylating agent.

The required *N*-substituted 2- and 4-hydroxycarbazoles **3** and **4** were prepared in one step (27–64% yields) according to this convenient procedure described for *N*-methyl and *N*-(prop-2-enyl)-2-hydroxycarbazoles **4a** and **4c** [29].

Twenty-four new oxazino[5,6-*c*]carbazoles **5** (Table 1) were then prepared by a Mannich type condensation of compounds **3** with 1.5 equivalent of various primary amines and 3 equivalents of formaldehyde (Scheme 2). Concerning the order of addition of the reactants, amine and formaldehyde are allowed to react first for 30 min at 0 °C and then combined with the hydroxycarbazole substrate. In the case of the reaction with *p*-methylbenzylamine, the aminometylating imine intermediate has been isolated and characterized.

The aminomethylation occurs in the *ortho* position as described for phenols and some other hydroxy heterocycles [30]. The intermediate Mannich bases are then cyclized by reaction with formaldehyde and lead to tetracyclic oxazinocarbazoles **5**.

The Mannich base is a secondary amine, and may also undergo an aminomethylation reaction with a second molecule of hydroxycarbazole substrate in the presence of formaldehyde. This process was observed only when 2-(aminomethyl)pyridine was used as the primary amine. Dimers **6** were obtained in small or moderate amount.

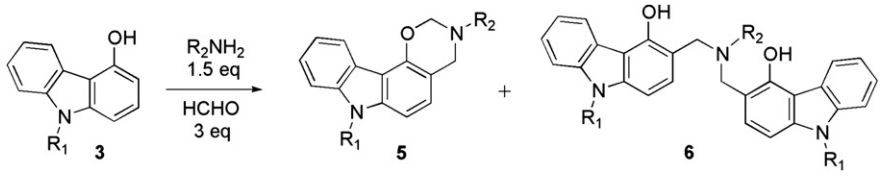
Some other amines, such as tryptamine, cyclohexylamine, 4-aminopyridine, 2,5-dimethoxyaniline, 2-aminobenzothiazole and 2-methoxyethylamine, were used as starting materials but only trace oxazinocarbazoles and a large amount of degradation products were observed.

Similarly, seven oxazino[6,5-*b*]carbazoles **7** and five oxazino [5,6-*a*]carbazoles **8** were obtained from *N*-methyl-2-hydroxycarbazole **4a** (Scheme 3). The product of dimerization **9** was obtained with a 13% yield only in the case of R = pyridin-2-ylmethyl. Its regioisomer corresponding to the aminomethylation at C1 of the carbazole nucleus was not detected.

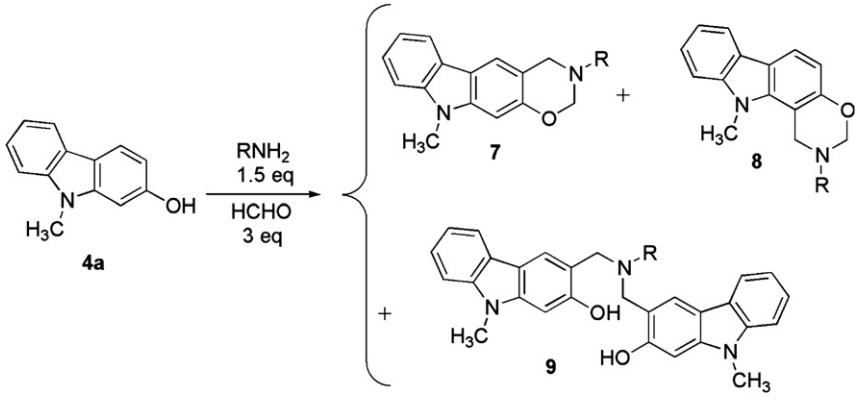
The Mannich type reaction of **4a** with the primary amines and formaldehyde led to a mixture of the two regioisomers **7** and **8** (Tables 2 and 3), which were isolated by chromatography, except in the case of aniline and 1,3-benzothiazol-2-ylmethanamine which led only to compounds **7**. The regioisomeric ratio was 1:2 in favor of **8** in the case of prop-2-en-1-amine (**7a**, **8a**), 1-phenylmethanamine (**7b**, **8b**), and 3-methylbut-2-en-1-amine (**7c**, **8c**), which correspond to a regioselective aminomethylation in the more hindered *ortho* position. Compounds **7e** and **8e** (R = pyridin-2-ylmethyl) were obtained with similar yields, and the regioisomeric ratio for **7d**/**8d**, obtained with the bulky amine 1-phenylethanamine, was 2:1 in favour of **7**. Identification of the regioisomeric oxazinocarbazoles was established from their ^{1}H NMR 300 MHz spectra.

3. Results and discussion

The antiproliferative activity of our compounds was evaluated with an in vitro assay performed on five human tumour cell lines: CEM (a T cell leukemia cell line), Jurkat (an acute T cell leukemia), Raji (Burkitt's lymphoma), MCF-7 (breast cancer cells) and Caco-2 (colorectal cancer). The activity was evaluated by measuring the levels of surviving cells after incubation for 72 h with the test samples, using the WST-1 colorimetric assay, based on the ability of metabolically active cells to convert the pink WST-1 to a yellow

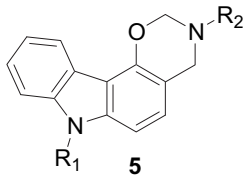


Scheme 2. Synthesis of oxazino[5,6-*c*]carbazole derivatives **5** and dimers **6**.

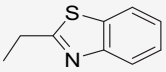
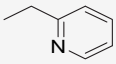
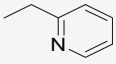
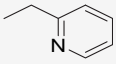


Scheme 3. Synthesis of oxazino[6,5-*b*]carbazole **7**, oxazino[5,6-*a*]carbazoles derivatives **8** and dimer **9**.

Table 1
Structure and antiproliferative activities of oxazino[5,6-*c*]carbazoles **5** against five tumor cell lines (CEM, Jurkat, Raji, MCF-7, Caco-2) at 100 μM.



5

Compound	R_1	R_2	Yield %	CEM	Jurkat	Raji	MCF-7	Caco-2
5a	CH_3	$CH_2CH{=}CH_2$	56	4.5	8.5	9.5	35	5
5b	CH_3	$CH_2C_6H_5$	84	10	87	80	87	100
5c	CH_3	CH_2-*p*$CH_3C_6H_4$	96	55.5	ND[a]	ND	42.5	ND
5d	CH_3	$CH(CH_3)C_6H_5$	35	40	80.5	84	100	100
5e	CH_3	C_6H_5	80	25	74.5	77	100	100
5f	CH_3		43	36	63	100	33	25.5
5g	CH_3		89	55.5	55	100	68	47
5h	C_2H_5	$CH_2CH{=}CH_2$	87	4	6.5	4.5	10	34
5i	C_2H_5	$CH_2C_6H_5$	68	31.5	ND	ND	64	46
5j	C_2H_5	CH_2-*p*$CH_3C_6H_4$	65	15	49.5	31.5	85.5	93
5k	C_2H_5	$CH(CH_3)C_6H_5$	79	21	ND	ND	86.5	59
5l	C_2H_5	C_6H_5	57	58.5	55	84	100	100
5m	C_2H_5		27	23	82	100	52	16
5n	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	75	2	2.5	19.5	43	12
5o	$CH_2CH{=}CH_2$	$CH_2C_6H_5$	66	5.5	67	100	55.5	56.5
5p	$CH_2CH{=}CH_2$	CH_2-*p*$CH_3C_6H_4$	33	9	3.5	9.5	66	54.5
5q	$CH_2CH{=}CH_2$	$CH(CH_3)C_6H_5$	29	ND	ND	ND	ND	ND
5r	$CH_2CH{=}CH_2$	C_6H_5	63	17	13.5	49.5	83	54.5
5s	$CH_2CH{=}CH_2$		22	14.5	12.5	31.5	47.5	55.5
5t	$CH_2CH{=}C(CH_3)_2$	$CH_2CH{=}CH_2$	51	4	2	3.5	2	39
5u	$CH_2CH{=}C(CH_3)_2$	$CH_2C_6H_5$	54	5.5	7.5	7	27.5	53
5v	$CH_2CH{=}C(CH_3)_2$	CH_2-*p*$CH_3C_6H_4$	23	11.5	13.5	11	41.5	48.5
5w	$CH_2CH{=}C(CH_3)_2$	$CH(CH_3)C_6H_5$	32	ND	ND	ND	ND	ND
5x	$CH_2CH{=}C(CH_3)_2$	C_6H_5	42	10.5	7.5	17	49	60

Activity was evaluated through the percentage of living cells after a 72 h incubation (100 indicates no activity).
[a] Not determined.

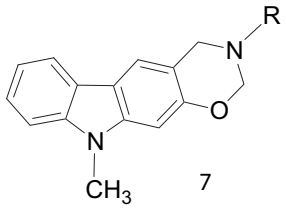
formazan product, which is spectrophotometrically quantifiable. All activities were compared to etoposide as a positive control; whose percentage of proliferation was evaluated at 100 μM on all five human tumour cell lines: CEM (0%), Jurkat (0%), Raji (0%), MCF-7(3%) and Caco-2(11%).

3.1. Primary screening at 100 μM

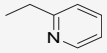
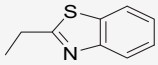
The screening for the most active compounds was performed at 100 μM on all five tumour cell lines. The results for oxazino[5,6-*c*] carbazoles **5** are reported in Table 1 and show that, from a structure-activity relationship's point of view of, the most active derivatives in this series display an allyl or prenyl group in the positions R_1 or R_2, which is the case of compounds **5a**, **5h**, **5n**, **5p**, **5t**, **5u**, **5v** and **5x**. In terms of cell line sensitivity, the higher activities were observed in the leukemia lines CEM, Jurkat and Raji. However, some derivatives showed a comparable activity on other lines. Derivatives **5a** and **5n** were also active on the colorectal cancer line Caco-2 and derivatives **5h** and **5t** on the breast cancer line MCF-7.

As for the oxazino[6,5-*b*]carbazoles **7**, compound **7g** with a benzothiazolylméthyl group in the oxazine cycle was the least active of the series (Table 2). With the sensitivity of cell lines to the structures **7** in perspective, the best activities were mainly expressed against the lines of type T-cell leukemia lines, CEM and Jurkat and to a lesser degree against the MCF-7 and Caco-2 lines. The comparison of data in Tables 1 and 2, show that the proliferation rates obtained with oxazino[6,5-*b*]carbazoles **7**, that are characterized by a linear sequence of four cycles, were lower than those observed for the oxazino[5,6-*c*]carbazoles **5** which have a rather angular structure.

Table 2
Structure and antiproliferative activities of oxazino[6,5-*b*]carbazoles **7** against five tumor cell lines (CEM, Jurkat, Raji, MCF-7, Caco-2) at 100 μM.



7

Compound	R	yield %	CEM	Jurkat	Raji	MCF-7	Caco-2
7a	$CH_2CH{=}CH_2$	31	1.5	1.5	10.5	1.5	2.5
7b	$CH_2C_6H_5$	25	0.5	1	11	3	1.5
7c	CH_2-*p*$CH_3C_6H_4$	21	1	2.5	27	27.5	6.5
7d	$CH(CH_3)C_6H_5$	42	0.5	1	18.5	0	2
7e		28	1.5	3.5	38	5	7.5
7f	C_6H_5	24	ND[a]	ND	ND	ND	ND
7g		31	34	17	78	79	51

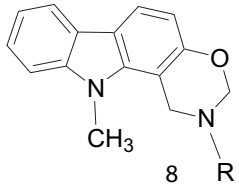
Activity was evaluated through the percentage of living cells after a 72 h incubation (100 indicates no activity).
[a] Not determined.

The results for oxazino[5,6-*a*]carbazoles **8** are shown in Table 3. They evidence a significant cytotoxic effect of all the compounds tested against CEM and Jurkat cells and to a lesser degree, against Caco-2 cells. In addition, derivatives **8c**–**e** were significantly active on the breast cancer cell line MCF-7. Generally, the activities of structures **8** were quite similar to those of structure **7**.

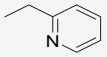
The results for dimers **6** and **9** are displayed in Table 4. They show a minimal activity of these structures on the five cancer cell lines, with the exception of derivative **9**, which was the most active against the T-cell leukemia lines CEM and Jurkat, the breast cancer MCF-7, and the colorectal cancer Caco-2.

Consequently, we were able to select ten molecules with an antiproliferative activity at 100 μM, comparable to the reference molecule etoposide. They were further subjected to a dose-dependent study.

Table 3
Structure and antiproliferative activities of oxazino[5,6-*a*]carbazoles **8** against five tumor cell lines (CEM, Jurkat, Raji, MCF-7, Caco-2) at 100 μM.



8

Compound	R	Reaction yield %	CEM	Jurkat	Raji	MCF-7	Caco-2
8a	$CH_2CH{=}CH_2$	61	1.5	2	60.5	62	5
8b	$CH_2C_6H_5$	51	1.5	5.5	31.5	60.5	6
8c	CH_2-*p*$CH_3C_6H_4$	42	1	1.5	12	1.5	3
8d	$CH(CH_3)C_6H_5$	21	0.5	0.5	19.5	0	5
8e		32	1	2	23	1.5	7

Activity was evaluated through the percentage of living cells after a 72 h incubation (100 indicates no activity).

3.2. Dose–response relationship

We studied the dose-response relationships of the ten most active compounds belonging to the main three series. Their effect was evaluated at a dose-range of 12.5 μM–100 μM and expressed as the concentration required to inhibit the growth of 50% of the cultured cells (IC_{50}, μMol L^{-1}) (Table 5).

In series **5**, we evaluated the activity of four compounds **5a** (R_1 = Me, R_2 = allyl), **5h** (R_1 = Et, R_2 = allyl), **5t** (R_1 = prenyl, R_2 = allyl) and **5u** (R_1 = prenyl, R_2 = benzyl). The antiproliferative effect of **5a** and **5h** was evaluated against the leukemic cell lines (CEM, Jurkat and Raji) and the colorectal cancer cell line Caco-2. The antiproliferative effect of **5t** and **5u** was evaluated against the five tumor cells.

Among these compounds **5t** was the most active on the CEM, Raji and Jurkat cell lines. Comparing the structure of **5t** with **5a**, **5h** and **5u**, it appeared that the association of a prenyl group at the N-position of the indole ring and an allyl group at the N-position of the oxazine ring resulted in the enhancement of the antiproliferative activity.

In series **7**, compounds **7a** (R = allyl), **7c** (R = 4-methylbenzyl), **7d** (R = α-methylbenzyl) and **7e** (R = pyridin-2-ylmethyl) were tested against the five tumor cells. The best activity was observed for **7e** on the CEM, Raji and Jurkat cell lines. The pyidin-2-ylmethyl group at the N-position of the oxazine ring resulted in the enhancement of the antiproliferative activity.

Finally among the oxazino[5,6-*a*]carbazoles series **8**, compound **8b** (R = benzyl) was evaluated against all the five cell lines and **8e** (R = pyridin-2-ylmethyl group) was evaluated against the CEM, Jurkat and Caco-2 cell lines. Both **8b** and **8e** showed a weak activity.

4. Conclusion

Forty-one compounds in four series of oxazinocarbazoles and *N*, *N*-bis(carbazolylmethyl)amines were synthesized and evaluated for their antiproliferative activity against five cancer cell lines.

In the primary screening studies at 100 μM, our data indicate that dimers **6** and **9** are less active than the oxazinocarbazole structures **5**, **7** and **8**. For the oxazinocarbazoles **5**, it seems that the presence of a prenyl or allyl substituent on the N-atom of the indole or the oxazine nucleus is potentially of interest to obtain antiproliferative activity. In terms of cell line sensitivity, best responses were observed against the leukemia cell lines CEM, Jurkat and Raji. In series **7** and **8**, all the tested compounds showed a significant antiproliferative activity at 100 μM, except for **7g**.

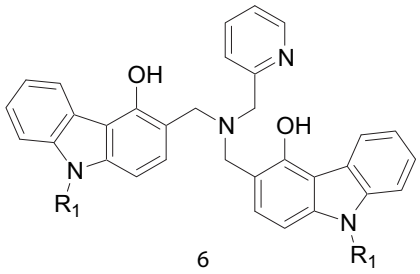
On the other hand, the dose–response relationship studies of the most active compounds evidenced that the best antiproliferative activity was observed for **5t** and **7e** against the leukemic cell lines CEM, Jurkat and Raji with IC_{50} values around 12 μM.

5. Experimental section

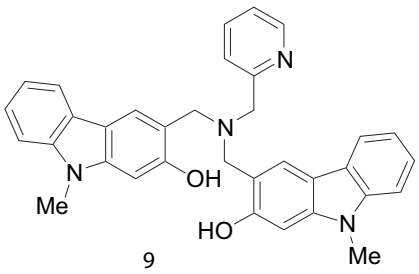
5.1. Chemistry

Melting points were determined on a Büchi 510 capillary apparatus. The IR spectra (KBr discs) were recorded on a Perkin–Elmer 1310 spectrometer. The ^1H and ^{13}C NMR spectra were recorded at 300 MHz on a Brücker AM 300 spectrometer. Chemical shifts are expressed in ppm (δ) downfield from internal tetramethylsilane. The following abbreviations are used: s: singlet; d: doublet; t: triplet; dd: doubled doublet; q: quartet; m: multiplet; C quat: quaternary carbons. The mass spectra were performed by direct ionization (EI or CI) on a ThermoFinnigan MAT 95 XL

Table 4
Structure and antiproliferative activities of dimers **6** and **9** against five tumor cell lines (CEM, Jurkat, Raji, MCF-7, Caco-2) at 100 μM.



6



9

Compound	R1	Reaction yield %	CEM	Jurkat	Raji	MCF-7	Caco-2
6a	CH_3	25	87.5	72	100	98.5	54
6b	C_2H_5	20	53	97.5	100	89	35.5
6c	$CH_2CH{=}CH_2$	22	78.5	92	95.5	94.5	94
6d	$CH_2CH{=}C(CH_3)_2$	13	100	100	100	100	100
9		13	9	8.5	70	24.5	10.5

Activity was evaluated through the percentage of living cells after a 72 h incubation (100 indicates no activity).

apparatus. Elemental analysis was performed at the Centre de Microanalyse, CNRS, Solaize, France.

*5.1.1. General procedure for the preparation of N-substituted 2- or 4-hydroxycarbazoles (**3**) and (**4**)*

In a dried tricol containing 750 mg of sodium hydride 60% NaH (18.75 mmol) under an argon atmosphere, 1.4 g of hydroxycarbazole (7.5 mmol) dissolved in 1.1 mL of DMF and 15 mL of anhydrous THF were added dropwise. Within 10 min, the alkylating agent (8.25 mmol) was added. The mixture was kept at room temperature and under an argon atmosphere for 2 h. The residue was hydrolyzed and neutralized with 15% HCl. After extraction with methylene chloride, the organic phase was dried over Na_2SO_4 and concentrated under vacuum. Column chromatography of the residue was eluted with 2% methanol in methylene chloride.

*5.1.1.1. 9-Allyl-9H-carbazol-4-ol (**3c**).* Alkylating agent: allyl iodide (0.76 mL), yield 44%, mp = 72 °C, IR (KBr): 3403 cm^{-1} (ν OH), ^1H NMR (δ, $CDCl_3$): 8.40 (d, 1H, J = 7.7 Hz, H-5); 7.53–7.26 (m, 4H, H aromat.); 7.00 (d, 1H, J = 8.3 Hz, H-3); 6.60 (d, 1H, J = 7.7 Hz, H-1); 6.10–6.21 (m, 1H, $CH_2CH{=}CH_2$); 5.67 (s, 1H, OH); 5.21–5.03 (m, 2H, $CH_2CH{=}CH_2$); 4.90 (d, 2H, J = 4.7 Hz, $CH_2CH{=}CH_2$); ^{13}C NMR (δ, $CDCl_3$,): 45.6; 116.9 (CH_2); 101.8; 105.1; 108.5; 119.5; 122.9; 125.1; 126.5; 132.4 (CH); 111.3 ;122.1; 139.9; 143.6; 152.1 (C quat); Anal Calcld for $C_{15}H_{13}NO$: C, 80.69; H, 5.87;N, 6.27; Found: C, 80.56; H, 6.08; N, 6.09.

*5.1.1.2. 9-Prenyl-9H-carbazol-4-ol (**3d**).* Alkylating agent: prenyl bromide (0.96 mL), yield 26%; mp = 77 °C, IR (KBr): 3419 cm^{-1} (ν OH); ^1H NMR (δ, $CDCl_3$): 8.34 (d, 1H, J = 7.7 Hz, H-5); 7.50–7.26 (m, 4H, H aromat.); 7.00 (d, 1H, J = 8.3 Hz, H-1); 6.58 (d, 1H, J = 7.9 Hz, H-3); 5.45 (s, 1H, OH); 5.29 (t, 1H, J = 6.2 Hz, $CH_2CH{=}C(CH_3)_2$); 4.89 (d, 2H, J = 6.2 Hz, $CH_2CH{=}C(CH_3)_2$); 1.94 (s, 3H, CH_3); 1.73 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 18.3; 25.7 (CH_3); 41.4 (CH_2); 101.8; 104.9; 108.5; 119.3; 120.1; 122.9; 125.0; 126.4 (CH); 111.3; 122.1; 135.2; 139.8; 142.3; 152.1 (C quat); Anal Calcld for: $C_{17}H_{17}NO$, 0.1 H_2O: C, 80.66; H, 6.84; N, 5.53; Found C, 80.69; H, 6.91; N, 5.24.

*5.1.2. General procedure for the preparation of oxazino[5,6-c] carbazoles (**5**) and dimers (**6**)*

A solution containing 1.5 mmol of primary amine and 3 mmol of formaldehyde dissolved in 10 mL methanol was stirred for 30 minutes at 0 °C. A solution containing 1 mmol of hydroxycarbazole-*N*-alkylated dissolved in 10 ml of methanol was added dropwise. The mixture was then stirred at room temperature (24 h). At the end of the reaction, the solvent was evaporated under vacuum and the residue was purified by flash chromatography with the suitable eluting agent.

*5.1.2.1. 3-Allyl-7-methyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5a**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl Acetate/Petroleum ether: 3/7); yield 56%, mp = 102 °C, IR (KBr): 1626, 1603, 1581 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 8.30 (d, 1H, J = 7.7 Hz, H-11); 7.44 (m, 1H, H-9 or H-10); 7.36 (d, 1H, J = 8.3 Hz, H-8); 7.23 (m, 1H, H-9 or H-10); 7.04 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.94 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.21 (m, 1H, $CH_2CH{=}CH_2$); 5.22 (m, 2H, $CH_2CH{=}CH_2$); 5.14 (s, 2H, H-2); 4.17

Table 5
In vitro antiproliferative activity (IC_{50} in μM)[a].

Compound	IC_{50} (μM)				
	CEM	Jurkat	Raji	Caco-2	MCF-7
5a	35.7 ± 11.5	69.0 ± 8.6	43.6 ± 8.1	32.1 ± 9.5	ND[b]
5h	44.0 ± 26.7	75.0 ± 0.0	43.6 ± 8.0	25.2 ± 0.2	ND
5t	11.8 ± 0.5	11.8 ± 0.5	11.9 ± 0.8	43.8 ± 8.9	37.0 ± 17.8
5u	37.6 ± 17.1	55.5 ± 8.1	39.3 ± 0.9	75.0 ± 0.0	75.0 ± 0.0
7a	50.1 ± 0.2	50.0 ± 0.2	56.2 ± 8.6	32.1 ± 9.8	62.6 ± 16.7
7c	68.8 ± 5.7	71.1 ± 6.7	72.8 ± 2.2	57.4 ± 10.5	75.0 ± 0.0
7d	25.2 ± 0.2	37.3 ± 12.7	57.9 ± 11.4	37.7 ± 17.3	62.5 ± 17.7
7e	16.5 ± 7.0	11.5 ± 0.6	12.1 ± 0.5	43.7 ± 8.8	56.4 ± 26.3
8b	70.8 ± 7.2	74.2 ± 1.4	75.0 ± 0.0	62.5 ± 17.7	72.1 ± 4.1
8e	74.3 ± 1.0	75.0 ± 0.0	ND	68.8 ± 0.1	ND

[a] Statistical analysis and IC_{50} values determination were performed using Microsoft Excel and nonlinear regression on Sigma Plot 11.0 (Systat Software, Inc. SigmaPlot for Windows).
[b] Not determined.

(s, 2H, H-4); 3.81 (s, 3H, CH_3); 3.49 (d, 2H, $J = 6.4$ Hz, $CH_2CH{=}CH_2$); ^{13}C NMR (δ $CDCl_3$): 29.2 (CH_3); 49.6; 54.6; 82.6; 118.25 (CH_2); 101.0; 107.9; 119.0; 123.1; 124.8; 125.1; 135.4 (CH); 109.0; 111.0; 121.9; 140.5; 141.4; 150.3 (C quat); Anal Calcld for: $C_{18}H_{18}N_2O$, 0.05 H_2O: C, 77.42; H, 6.53; N, 10.03; Found: C, 77.43; H, 6.67; N, 9.99.

*5.1.2.2. 3-Benzyl-7-methyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5b**).* Purified by column chromatography on silica gel yielded a pink solid (Eluent = Ethyl Acetate/Petroleum ether: 3/7), yield 84%, mp = 139 °C, IR (KBr): 1630, 1603, 1580 cm^{-1}; ^1H NMR (δ $CDCl_3$): 8.22 (d, 1H, $J = 7.7$ Hz, H-11); 7.35–7.14 (m, 8H, H aromat.); 6.91 (d, 1H, $J = 8.3$ Hz, H-5 ou H-6); 6.84 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.05 (s, 2H, H-2); 4.04 (s, 2H, H-4); 3.93 (s, 2H, CH_2Ph); 3.71 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.2 (CH_3); 49.6; 55.6; 82.8 (CH_2); 101.0; 107.9; 119.1; 123.1; 124.8; 125.2; 127.4; 128.5 (2C); 129.1 (2C) (CH); 109.0; 111.0; 121.9; 138.5; 140.5; 141.4; 150.3 (C quat); Anal Calcld for: $C_{22}H_{20}N_2O$, 0.33 H_2O: C, 79.03; H, 6.23; N, 8.38; Found: C, 79.03; H, 6.34; N; 8.26.

*5.1.2.3. 7-Methyl-3-(4-methylbenzyl)-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5c**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl Acetate/Petroleum ether: 3/7), yield 96%, mp = 113 °C; IR (KBr): 1632, 1603, 1580, 1512 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 8.37 (d, 1H, $J = 7.7$ Hz, H-11); 7.52–7.21 (m, 7H, H aromat.); 7.05 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 6.98 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.20 (s, 2H, H-2); 4.18 (s, 2H, H-4); 4.03 (s, 2H, CH_2Ar); 3.86 (s, 3H, NCH_3); 2.42 (s, 3H, ArCH_3); ^{13}C NMR (δ, $CDCl_3$): 21.23; 29.2 (CH_3); 49.5; 55.3; 82.7 (CH_2); 100.9; 107.9; 119.0; 123.1; 124.8; 125.2; 127.4; 129.1 (2C); 129.2 (2C) (CH); 109.0; 111.0; 121.9; 135.4; 137.0; 140.5; 141.4; 150.3 (C quat); Anal Calcld for: $C_{23}H_{22}N_2O$: C, 80.67; H, 6.48; N, 8.18; Found: C, 80.30; H, 6.44; N, 7.95.

*5.1.2.4. 7-Methyl-3-(α-methylbenzyl)-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5d**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl Acetate/Petroleum ether: 3/7); yield 35%, mp = 100 °C;IR (KBr): 1629, 1604, 1580 cm^{-1}; ^1H NMR(δ, $CDCl_3$): 8.21 (d, 1H, $J = 7.7$ Hz, H-11); 7.48–7.21 (m, 8H, H aromat.); 6.97 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 6.91 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.36 (d, 1H, $J = 10.2$ Hz, H-2); 5.13 (d, 1H, $J = 10.2$ Hz, H-2); 4.31 (d, 1H, $J = 16.4$ Hz, H-4); 4.11 (q, 1H, $J = 6.6$ Hz, $CH(CH_3)$Ph); 3.95 (d, 1H, $J = 16.4$ Hz, H-4); 3.82 (s, 3H, NCH_3); 1.53 (d, 3H, $J = 6.6$ Hz, CH(CH_3)Ph); ^{13}C (δ, $CDCl_3$): 21.7, 29.2 (CH_3); 48.9; 80.6 (CH_2); 57.5; 100.7; 107.9; 119.0; 123.1; 124.8; 125.1; 127.2; 127.4 (2C); 128.6 (2C) (CH); 109.5; 111.1; 121.9; 140.5; 141.4; 144.9; 151.0 (C quat); Anal Calcld for: $C_{23}H_{22}N_2O$: C, 80.67; H, 6.48; N, 8.18; Found: C, 80.51; H, 6.45; N, 7.98.

*5.1.2.5. 7-Methyl-3-phenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5e**).* Purified by column chromatography on silica gel yielded a pale brown solid (Eluent = Ethyl Acetate/Petroleum ether: 7/3); yield 80%, mp = 158 °C; IR (KBr): 1627, 1599 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.29 (d, 1H, $J = 7.5$ Hz, H-11); 7.42–7.17 (m, 7H, H aromat.); 7.01 (d, 1H, $J = 8.3$ Hz, H-8); 6.89 (m, 2H, H-5 et H-6); 5.60 (s, 2H, H-2); 4.77 (s, 2H, H-4); 3,74 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.2 (CH_3); 50.7; 80.0 (CH_2); 101.2; 107.9; 118.7 (2C); 119.0; 121.4; 123.1; 124.2; 124.9; 129.3 (2C) (CH); 111.0; 111.4; 121.7; 140.5; 141.4; 148.8; 150.5 (C quat); Anal Calcld for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91, Found: C, 80.40; H, 5.90; N, 8.77.

*5.1.2.6. 7-Methyl-3-pyridin-2-ylmethyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5f**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl Acetate/Petroleum ether: 7/3); yield 43%, mp = 135 °C; IR (KBr): 1627, 1602, 1579 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 8.64 (d, 1H, $J = 4.3$ Hz, H-6′); 8.33 (d, 1H, $J = 7.7$ Hz, H-11); 7.68 (m, 1H, H-4′); 7.47–7.18 (m, 5H, H aromat.); 7.01 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 6.93 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.20 (s, 2H, H-2); 4.18 (s, 4H, H-4 et CH_2Py); 3.80 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.2 (CH_3); 50.2; 57.5; 83.3 (CH_2); 101.1; 107.9; 119.1; 122.3; 123.2 (2C); 124.9; 125.2; 136.6; 149.8 (CH); 109.0; 111.2; 121.9; 140.5; 141.5; 150.3; 158.7 (C quat); HRMS-EI calculated for $C_{21}H_{19}N_3O$, $M^+ = 329.1528$; Found, $M^+ = 329.1534$.

*5.1.2.7. 3-Benzothiazol-2-ylmethyl-7-methyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5g**).* Purified by column chromatography on silica gel yielded a pink solid (Eluent = CH_2Cl_2); yield 89%, mp = 195 °C; IR (KBr): 1629, 1603, 1578, 1519 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.20 (d, 1H, $J = 7.7$ Hz, H-11); 7.88 (d, 1H, $J = 7.5$ Hz, H-4′); 7.81 (d, 1H, $J = 7.7$ Hz, H-7′); 7.40–7.10 (m, 5H, H aromat.); 6.90 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 6.85 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.15 (s, 2H, H-2); 4.37 (s, 2H, H-4); 4.18 (s, 2H, CH_2Ar); 3.72 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.3 (CH_3); 50.6; 54.6; 83.3 (CH_2); 101.5; 108.0; 119.2; 121.8; 121.9; 123.1; 123.2; 125.0; 125.1; 125.2; 126.1 (CH); 108.5; 111.2; 135.4; 140.6; 141.6; 150.0; 153.7; 172.3 (C quat); Anal Calcld for: $C_{23}H_{19}N_3OS$, 0.55 H_2O: C, 69.88; H, 5.12; N, 10.63; Found: C, 69.95; H, 5.15; N, 10.20.

*5.1.2.8. 3-Allyl-7-ethyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5h**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Acetone/Petroleum ether: 2/8); yield 87%, mp = 76 °C; IR (KBr): 1628, 1604, 1579, 1488 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.35 (d, 1H, $J = 7.7$ Hz, H-11); 7.49–7.44 (m, 1H, H-9 or H-10); 7.40 (d, 1H, $J = 8.1$ Hz, H-8); 7.28–7.20 (m, 1H, H-9 or H-10); 7,06 (d, 1H, $J = 8,3$ Hz, H-5 or H-6); 6,98 (d, 1H, $J = 8,3$ Hz, H-5 or H-6); 6.01 (m, 1H, $CH_2CH{=}CH_2$); 5.27 (m, 2H, $CH_2CH{=}CH_2$); 5.17 (s, 2H, H-2); 4.36 (q, 2H, $J = 7.2$ Hz, CH_2CH_3); 4.21 (s, 2H, H-4); 3.54 (d, 2H, $J = 6.4$ Hz, $CH_2CH{=}CH_2$); 1.45 (t, 3H, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 13.9 (CH_3); 37.6; 49.5; 54.6; 82.5; 118.25 (CH_2); 101.0; 107.9; 118.9; 123.3; 124.7; 125.0; 135.4 (CH); 108.9; 111.2; 122.0; 139.4; 140.3; 150.4 (C quat); Anal Calcld for: $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58; Found: C, 77.95; H, 7.06; N, 9.46.

*5.1.2.9. 3-Benzyl-7-ethyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5i**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate/Petroleum ether: 2/8); yield 68%, mp = 132 °C; IR (KBr): 1630, 1602, 1578, 1495 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.23 (d, 1H, $J = 7.9$ Hz, H-11); 7.37–7.17 (m, 7H, H aromat.); 7.13 (m, 1H, H-9 or H-10); 6.90 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 6.85 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.05 (s, 2H, H-2); 4.23 (q, 2H, $J = 7.2$ Hz, CH_2CH_3); 4.04 (s, 2H, H-4); 3.93 (s, 2H, CH_2Ph); 1.32 (t, 3H, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 13.9 (CH_3); 37.6; 49.6; 55.6; 82.8 (CH_2); 101.0; 107.9; 118.9; 123.3; 124.8; 125.1; 127.4; 128.5 (2C); 129.1 (2C) (CH); 108.9; 111.2; 122.0; 138.6; 139.4; 140.4; 150.4 (C quat); Anal Calcld for: $C_{23}H_{22}N_2O$, 0.2 H_2O: C, 79.83; H, 6.52; N, 8.09; Found: C, 79.91; H, 6.64; N, 7.89.

*5.1.2.10. 7-Ethyl-3-(4-methylbenzyl)-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5j**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Acetone/Petroleum ether: 2/8); yield 65%, mp = 131 °C; IR (KBr): 1631, 1602, 1578, 1513 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.36 (d, 1H, $J = 7.7$ Hz, H-11); 7.49–7.18 (m, 7H, H aromat.); 7.03 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 6.97 (d, 1H, $J = 8.3$ Hz, H-5 or H-6); 5.17 (s, 2H, H-2); 4.35 (q, 2H, $J = 7.2$ Hz, CH_2CH_3); 4.16 (s, 2H, H-4); 4.02 (s, 2H, CH_2Ar); 2.40 (s, 3H, ArCH_3); 1.45 (t, 3H, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 14.0; 21.3 (CH_3); 29.0; 37.7; 49.6; 55.3; 82.8 (CH_2); 101.0; 107.9; 119.0; 123.3; 124.8; 125.2; 129.1; 129.2 (CH); 109.0; 111.2; 122.1; 135.5; 137.0; 139.5; 140.4; 150.0; 150.5 (C quat); Anal Calcld for: $C_{24}H_{24}N_2O$, 0.2 H_2O: C, 80.05; H, 6.83; N, 7.78; Found: C, 79.85; H, 6.72; N, 7.74.

*5.1.2.11. 7-Ethyl-3-(alpha-methylbenzyl)-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5k**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Acetone/Petroleum ether: 2/8); yield 79%, mp = 102 °C; IR (KBr): 1630, 1600, 1580, 1485 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.21 (d, 1H, J = 7.7 Hz, H-11); 7.36–7.09 (m, 8H, H aromat.); 6.84 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.81 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.25 (d, 1H, , J = 10.2 Hz, H-2); 5.02 (d, 1H, J = 10.2 Hz, H-2); 4.25 (q, 2H, J = 7.2 Hz, CH_2CH_3); 4.19 (d, 1H, J = 16 Hz, H-4); 4.01 (q, 1H, J = 6.6 Hz, $CH(CH_3)Ph$); 3.83 (d, 1H, J = 16 Hz, H-4); 1.42 (d, 3H, J = 6.6 Hz, $CH(CH_3)Ph$); 1.31 (t, 3H, J = 7.2 Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 13.9; 21.7 (CH_3); 37.6; 48.9; 80.5 (CH_2); 57.5; 100.8; 107.9; 118.9; 123.3; 124.7; 125.0; 127.2; 127.4 (2C); 128.6 (2C) (CH); 109.3; 111.1; 122.0; 139.4; 140.3; 144.9; 151.1 (C quat); Anal Calcld for: $C_{24}H_{24}N_2O$, 0.15 H_2O: C, 80.26; H, 6.82; N, 7.80; Found: C, 80.38; H, 7.13; N, 7.53.

*5.1.2.12. 7-Ethyl-3-phenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5l**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 2/8); yield 57%, mp = 109 °C; IR (KBr): 1630, 1600, 1580, 1498 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.23 (d, 1H, J = 7.7 Hz, H-11); 7.34–7.10 (m, 7H, H aromat.); 6.97 (d, 1H, J = 8.1 Hz, H-5 or H-6); 6.84 (d, 1H, J = 8.1 Hz, H-5 or H-6); 6.80 (d, 1H, J = 6.9 Hz, H-8); 5.52 (s, 2H, H-2); 4.70 (s, 2H, H-4); 4.19 (q, 2H, J = 7.2 Hz, CH_2CH_3); 1.25 (t, 3H, J = 7.2 Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 13.9 (CH_3); 37.6; 50.8; 80.0 (CH_2); 101.2; 107.9; 118.7 (2C); 118.9; 121.5; 123.3; 124.1; 124.8; 129.3 (2C) (CH); 110.0; 111.6; 121.9; 139.4; 140.3; 148.8; 150.6 (C quat); Anal Calcld for: $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53; Found: C, 80.26; H, 6.26; N, 8.36.

*5.1.2.13. 7-Ethyl-3-pyridin-2-ylmethyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5m**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 7/3); yield 27%, mp = 132 °C; IR (KBr): 1627, 1590, 1581, 1568 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.64 (d, 1H, J = 4.3 Hz, H-6′); 8.34 (d, 1H, J = 7.7 Hz, H-11); 7.68 (m, 1H, H-4′); 7.46–7.19 (m, 5H, H aromat.); 7.01 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.95 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.20 (s, 2H, H-2); 4.33 (q, 2H, J = 7.4 Hz, CH_2CH_3); 4.19 (s, 4H, H-4 et CH_2Ar); 1.42 (t, 3H, J = 7.2 Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 13.9 (CH_3); 37.7; 50.2; 57.5; 83.3 (CH_2); 101.2; 107.9; 119.0; 122.1; 123.2; 123.3; 124.8; 125.1; 136.6; 149.8 (CH); 108.9; 111.3; 122.3; 139.5; 140.4; 150.4; 158.7 (C quat); Anal Calcld for: $C_{22}H_{21}N_3O$, 0.5 H_2O: C, 74.97; H, 6.29; N, 11.92; Found: C, 74.96; H, 6.09; N, 12.23.

*5.1.2.14. 3,7-Diallyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5n**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2); yield 75%, mp = 87 °C; IR (KBr): 1627, 1603, 1578, 1487 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.31 (d, 1H, J = 7.7 Hz, H-11); 7.41 (m, 1H, H-9 or H-10); 7.33 (d, 1H, J = 8.1 Hz, H-8); 7.23 (m, 1H, H-9 or H-10); 7.01 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.91 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.05–5.90 (m, 2H, $CH_2CH{=}CH_2$); 5.30–5.00 (m, 4H, $CH_2CH{=}CH_2$); 5.14 (s, 2H, H-2); 4.88–4.86 (m, 2H, $CH_2CH{=}CH_2$); 4.16 (s, 2H, H-4); 3.51–3.49 (m, 2H, $CH_2CH{=}CH_2$); ^{13}C NMR (δ, $CDCl_3$): 45.4; 49.6; 55.7; 82.7; 116.8; 118.3 (CH_2); 101.3; 108.3; 119.3; 123.3; 124.9; 125.1; 132.5; 135.5 (CH); 109.3; 111.3; 122.1; 139.9; 140.8; 150.4 (C quat); Anal Calcld for: $C_{20}H_{20}N_2O$: C, 78.92; H, 6.62; N, 9.20; Found: C, 78.72; H, 6.85; N, 9.28.

*5.1.2.15. 7-Allyl-3-benzyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5o**).* Crystallization from methanol yielded a green solid; yield 66%, mp = 118 °C; IR (KBr): 1627, 1602, 1582, 1493 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.24 (d, 1H, J = 7.5 Hz, H-11); 7.45–7.21 (m, 8H, H aromat.); 6.99 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.92 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.06–5.94 (m, 1H, $CH_2CH{=}CH_2$); 5.16 (s, 2H, H-2); 5.18–5.03 (m, 2H, $CH_2CH{=}CH_2$); 4.88 (d, 2H, J = 4.7 Hz, $CH_2CH{=}CH_2$); 4.14 (s, 2H, H-4); 4.04 (s, 2H, CH_2Ph); ^{13}C NMR (δ, $CDCl_3$): 45.5; 49.7; 55.7; 82.9; 116.9 (CH_2); 101.4; 108.3; 119.3; 123.3; 124.9; 125.3; 127.4; 128.6 (2C); 129.1 (2C); 132.5 (CH); 109.3; 111.3; 122.1; 138.6; 140.0; 150.4 (C quat); Anal Calcld for: $C_{24}H_{22}N_2O$: C, 81.33; H, 6.26; N, 7.90; Found: C, 81.13; H, 6.29; N, 7.94.

*5.1.2.16. 7-Allyl-3-(4-methylbenzyl)-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5p**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/Petroleum ether: 9/1); yield 33%, mp = 116 °C; IR (KBr): 1650, 1627, 1602, 1581 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.36 (d, 1H, J = 7.5 Hz, H-11); 7.44–7.18 (m, 7H, H aromat.); 7.00 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.92 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.07–5.94 (m, 1H, $CH_2CH{=}CH_2$); 5.16 (s, 2H, H-2); 5.19–5.04 (m, 2H, $CH_2CH{=}CH_2$); 4.88 (d, 2H, J = 4.5 Hz, $CH_2CH{=}CH_2$); 4.15 (s, 2H, H-4); 4.02 (s, 2H, CH_2Ar); 2.39 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 21.3 (CH_3); 45.5; 49.6; 55.4; 82.8; 116.8 (CH_2); 101.3; 108.3; 119.3; 123.3; 124.9; 125.3; 129.1 (2C); 129.2 (2C); 132.6 (CH); 109.3; 111.3; 122.2; 135.5; 137.0; 140.0; 140.9; 151.5 (C quat); Anal Calcld for: $C_{25}H_{24}N_2O$, 0.25 H_2O: C, 80.50; H, 6.62; N, 7.51; Found: C, 80.79; H, 6.58; N, 7.16.

*5.1.2.17. 7-Allyl-3-(α-methylbenzyl)-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5q**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/Petroleum ether: 8/2); yield 29%, mp = 67 °C; IR (KBr): 1627, 1604, 1581, 1487 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.20 (d, 1H, J = 7.7 Hz, H-11); 7.31–7.07 (m, 8H, H aromat.); 6.79 (d, 1H, J = 8.1 Hz, H-5 or H-6); 6.73 (d, 1H, J = 8.1 Hz, H-5 or H-6); 5.90–5.77 (m, 1H, $CH_2CH{=}CH_2$); 5.22 (d, 1H, J = 10.2 Hz, H-2); 4.97–4.87 (m, 3H, $CH_2CH{=}CH_2$ et H-2); 4.70 (d, 2H, J = 4.9 Hz, $CH_2CH{=}CH_2$); 4.14 (d, 1H, J = 16.2 Hz, H-4); 3.98 (q, 1H, J = 6.6 Hz, $CH(CH_3)Ph$); 3.78 (d, 1H, J = 16.2 Hz, H-4); 1.39 (d, 3H, J = 6.6 Hz, $CH(CH_3)Ph$); ^{13}C NMR (δ, $CDCl_3$): 21.8 (CH_3); 45.5; 48.9; 80.7; 116.8 (CH_2); 57.6; 101.1; 108.3; 119.3; 119.7; 123.3; 124.9; 125.1; 127.3; 127.5; 128.6; 132.6; 136.9 (CH); 109.8; 111.3; 122.2; 140.0; 140.8; 145.0; 151.2 (C quat); HRMS-CI calculated for $C_{25}H_{23}N_2O$, MH^+ = 369.1967, Found, MH^+ = 369.1971.

*5.1.2.18. 7-Allyl-3-phenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5r**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/Petroleum ether: 6/4); yield 63%, mp = 67 °C; IR (KBr): 1629, 1601, 1581, 1494 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.32 (d, 1H, J = 7.9 Hz, H-11); 7.44–7.20 (m, 7H, H aromat.); 7.07 (d, 1H, J = 8.3 Hz, H-8); 6.91 (m, 2H, H-5 et H-6); 6.03–5.90 (m, 1H, $CH_2CH{=}CH_2$); 5.63 (s, 2H, H-2); 5.16–4.97 (m, 2H, $CH_2CH{=}CH_2$); 4.85 (d, 2H, J = 4.7 Hz, $CH_2CH{=}CH_2$); 4.81 (s, 2H, H-4); ^{13}C NMR (δ, $CDCl_3$): 45.3; 50.7; 80.0; 116.7 (CH_2); 101.6; 108.3; 118.7 (2C); 119.3; 121.5; 123.3; 124.2; 125.0; 129.3 (2C); 132.4 (CH); 110.4; 111.6; 122.0; 139.9; 140.8; 148.8; 150.6 (C quat); HRMS-CI calculated for $C_{23}H_{21}N_2O$, MH^+ = 341.1654, Found, MH^+ = 341.1653.

*5.1.2.19. 7-Allyl-3-pyridin-2-ylmethyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazol (**5s**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/MeOH: 8/2); yield 22%, mp = 134 °C; IR (KBr): 1626, 1589, 1581, 1491 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.54 (d, 1H, J = 4.7 Hz, H-6′); 8.23 (d, 1H, J = 7.7 Hz, H-11); 7.58 (m, 1H, H-4′); 7.35–7.09 (m, 5H, H aromat.); 6.90 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.82 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.99–4.83 (m, 1H, $CH_2CH{=}CH_2$); 5.10 (s, 2H, H-2); 5.08–4.90 (m, 2H, $CH_2CH{=}CH_2$); 4.78 (d, 2H, J = 4.7 Hz, $CH_2CH{=}CH_2$); 4.09 (s, 4H, H-4 et CH_2Ar); ^{13}C NMR (δ, $CDCl_3$): 45.4; 50.1; 57.5; 83.4; 116.8 (CH_2); 101.5; 108.3; 119.3; 122.4; 123.3 (2C); 124.9; 125.3; 132.5; 136.7; 149.9 (CH); 109.2; 111.4; 122.1; 139.9; 140.9; 150.4; 158.7 (C quat); Anal Calcld for: $C_{23}H_{21}N_3O$, 0.25 H_2O: C, 76.75; H, 6.02; N, 11.67; Found: C, 76.64; H, 6.05; N, 11.75.

*5.1.2.20. 3-Allyl-7-prenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5t**).* Purified by column chromatography on silica gel yielded a green solid (Eluent = CH_2Cl_2); yield 51%, mp = 88 °C; IR (KBr): 1627, 1602, 1579, 1486 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.21 (d, 1H, J = 7.7 Hz, H-11); 7.32 (m, 1H, H-9 or H-10); 7.25 (d, 1H, J = 8. Hz, H-8); 7.12 (m, 1H, H-9 or H-10); 6.91 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.82 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.89–5.83 (m, 1H, CH_2CH=CH_2); 5.20–5.10 (m, 3H, CH_2CH=CH_2 et CH_2CH=$C(CH_3)_2$); 5.03 (s, 2H, H-2); 4.75 (d, 2H, J = 6.4 Hz, CH_2CH=CH_2); 4.7 (s, 2H, H-4); 3.40 (d, 2H, J = 6.2 Hz, CH_2CH=$C(CH_3)_2$); 1.82 (s, 3H, CH_3); 1.62 (s, 3H, CH_3); ^{13}C NMR i(δ, $CDCl_3$): 18.3; 25.7 (CH_3); 41.3; 49.6; 54.6; 82.6; 119.0 (CH_2); 101.3; 108.3; 118.2; 120.2; 123.3; 124.8; 125.0; 135.1 (CH); 109.0; 111.3; 122.1; 135.1; 139.8; 140.7; 150.5 (C quat); HRMS-EI calculated for $C_{22}H_{24}N_2O$, M^+ = 332.1889; Found, M^+ = 332.1893.

*5.1.2.21. 3-Benzyl-7-prenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5u**).* Purified by column chromatography on silica gel yielded a green solid (Eluent = Ethyl acetate /Petroleum ether: 3/7); yield 54%, mp = 121 °C; IR (KBr): 1628, 1602, 1580, 1493 cm^{-1}, ^1H NMR ($\delta\delta$, $CDCl_3$): 8,33 (d, 1H, J = 7,7 Hz, H-11); 7,45–7,20 (m, 8H, H aromat.); 6,99 (d, 1H, J = 8,1 Hz, H-5 or H-6); 6.93 (d, 1H, J = 8.1 Hz, H-5 or H-6); 5.28 (t, 1H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 5.15 (s, 2H, H-2); 4.87 (d, 2H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 4.14 (s, 2H, H-4); 4.04 (s, 2H, CH_2Ph); 1.93 (s, 3H, CH_3); 1.71 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 18.3; 25.7 (CH_3); 41.3; 49.7; 55.7; 82.9 (CH_2); 101.4; 108.3; 119.1; 120.2; 123.3; 124.8; 125.1; 127.4; 128.5 (2C); 129.1 (2C) (CH); 109.0; 111.3; 122.1; 135.1; 138.7; 139.9; 140.8; 150.4 (C quat); Anal Calcld for: $C_{26}H_{26}N_2O$: C, 81.64; H, 6.85; N, 7.32; Found: C, 81.56; H, 6.85; N, 7.32.

*5.1.2.22. 3-(4-Methylbenzyl)-7-prenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5v**).* Purified by column chromatography on silica gel yielded a green solid (Eluent = Ethyl acetate /Petroleum ether: 2/8); yield 23%, mp = 114 °C; IR (KBr): 1629, 1602, 1579, 1515 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.37 (d, 1H, J = 7.7 Hz, H-11); 7.48–7.19 (m, 7H, H aromat.); 7.02 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.95 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.31 (t, 1H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 5.17 (s, 2H, H-2); 4.88 (d, 2H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 4.16 (s, 2H, H-4); 4.02 (s, 2H, CH_2Ar); 2.40 (s, 3H, ArCH_3); 1.95 (s, 3H, CH_3); 1.74 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 18.3; 21.3; 25.7 (CH_3); 41.3; 49.6; 55.3; 82.8 (CH_2); 101.3; 108.3; 119.0; 120.2; 123.3; 124.8; 125.1; 129.1 (2C); 129.2 (2C) (CH); 109.0; 111.3; 122.1; 135.1; 135.6; 137.0; 139.9; 140.7; 150.5 (C quat); Anal Calcld for: $C_{27}H_{28}N_2O$: C, 81.78; H, 7.12; N, 7.06; Found: C, 81.78; H, 7.25; N, 6.77.

*5.1.2.23. 3-(α-Methylbenzyl)-7-prenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5w**).* Purified by column chromatography on silica gel yielded a green solid (Eluent = CH_2Cl_2); yield 32%, mp = 94 °C; IR (KBr): 1628, 1602, 1579, 1493 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.34 (d, 1H, J = 7.7 Hz, H-11); 7.56–7.12 (m, 8H, H aromat.); 6.96 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.91 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.38 (d, 1H, J = 10.2 Hz, H-2); 5.30 (t, 1H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 5.14 (d, 1H, J = 10.2 Hz, H-2); 4.87 (d, 2H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 4.33 (d, 1H, J = 16.2 Hz, H-4); 4.14 (q, 1H, J = 6.6 Hz, $CH(CH_3)$Ph); 3.96 (d, 1H, J = 16.2 Hz, H-4); 1.78 (s, 3H, CH_3); 1.73 (s, 3H, CH_3); 1.55 (d, 3H, J = 6.6 Hz, CH(CH_3)Ph); ^{13}C NMR (δ, $CDCl_3$): 18.3; 21.8; 25.7 (CH_3); 41.3; 48.9; 80.6 (CH_2); 57.6; 101.1; 108.3; 119.0; 120.2; 123.3; 124.8; 125.0; 127.3; 127.5 (2C); 128.6 (2C) (CH); 109.5; 111.2; 122.1; 135.1; 139.9; 140.7; 145.1; 151.2 (C quat); HRMS-CI calculated for $C_{27}H_{28}N_2O$, MH^+ = 397.2280, Found, MH^+ = 397.2291.

*5.1.2.24. 3-Phenyl-7-prenyl-2,3,4,7-tetrahydro[1,3]oxazino[5,6-c]carbazole (**5x**).* Purified by column chromatography on silica gel yielded a green solid (Eluent = Ethyl acetate /Petroleum ether: 2/8); yield 42%, mp = 106 °C; IR (KBr): 1626, 1600, 1578, 1495 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.32 (d, 1H, J = 7.7 Hz, H-11); 7.44–7.15 (m, 7H, H aromat.); 7.08 (d, 1H, J = 8.1 Hz, H-8); 6.94 (d, 1H, J = 8.3 Hz, H-5 or H-6); 6.94 (d, 1H, J = 8.3 Hz, H-5 or H-6); 5.63 (s, 2H, H-2); 5.30–5.23 (t, 1H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 4.86 (d, 2H, J = 6.4 Hz, CH_2CH=$C(CH_3)_2$); 4.81 (s, 2H, H-4); 1.92 (s, 3H, CH_3); 1.70 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 18.3; 25.7 (CH_3); 41.2; 50.8; 80.0 (CH_2); 101.6; 108.3; 117.8; 118.7; 119.0; 120.1; 121.0; 121.5; 123.3; 124.1; 124.9; 129.3 (CH); 110.1; 111.7; 122.0; 135.1; 139.8; 140.7; 148.9; 150.6 (C quat); HRMS-CI calculated for $C_{25}H_{25}N_2O$, MH^+ = 369.1967, Found, MH^+ = 369.1968.

*5.1.2.25. 3-{[(4-Hydroxy-9-methyl-9H-carbazol-3-ylmethyl)(pyridin-2-ylmethyl)amino]}-9-methyl-9H-carbazol-4-ol (**6a**).* Crystallization from methanol yielded an off-white solid; yield 25%, mp = 182 °C; IR (KBr): 1636, 1600, 1583 cm^{-1}; ^1H NMR (δ, DMSO-d_6): 11.56 (s, 2H, OH); 8.83 (d, 1H, J = 4.7 Hz, H-6′); 8.24 (d, 2H, J = 7.5 Hz, H-5); 7.91 (d, 1H, J = 7.7 Hz, H-4′); 7.52–7.15 (m, 10H, H aromat.); 7.03 (d, 2H, J = 8.1 Hz, H-1); 4.00 (s, 6H, CH_2-N); 3.80 (s, 6H, CH_3); ^{13}C NMR (δ, DMSO-d_6): 29.9 (2C) (CH_3); 55.7 (2C); 56.2 (CH_2); 100.8 (2C); 109.3 (2C); 119.5 (2C); 123.2 (2C); 123.8; 124.8; 125.4 (2C); 129.2 (2C); 139.0; 148.9 (CH); 111.8 (2C); 112.3 (2C); 122.5 (2C); 141.0 (2C); 143.0 (2C); 153.9 (2C); 157.2 (C quat); HRMS-CI calculated for $C_{34}H_{31}N_4O_2$, MH^+ = 526.2369, Found, MH^+ = 526.2371.

*5.1.2.26. 3-{[(9-Ethyl-4-hydroxy-9H-carbazol-3-ylmethyl)(pyridin-2-ylmethyl)amino]}-9-ethyl-9H-carbazol-4-ol (**6b**).* Crystallization from methanol yielded an off-white solid; yield 20%, mp = 178 °C; IR (KBr): 1634, 1601, 1583 cm^{-1}; ^1H NMR (δ, DMSO-d_6): 11.58 (s, 2H, OH); 8.82 (d, 1H, J = 4.7 Hz, H-6′); 8.24 (d, 2H, J = 7.7 Hz, H-5); 7.89 (m, 1H, H-4′); 7.52–7.13 (m, 10H, H aromat.); 7.02 (d, 2H, J = 8.1 Hz, H-1); 4.35 (q, 4H, J = 7.0 Hz, CH_2CH_3); 3.98 (s, 6H, NCH_2); 1.27 (t, 6H, J = 7.0 Hz, CH_2CH_3); ^{13}C NMR (δ, $CDCl_3$): 14.0 (2C) (CH_3); 37.7 (3C); 55.7 (2C) (CH_2); 99.5; 99.6; 101.2; 107.7 (2C); 119.0 (2C); 119.1; 122.9; 123.7; 124.6; 124.8; 124.9; 128.4; 137.9; 148.2 (CH); 91.5; 111.0; 112.3; 139.6 (2C); 139.7 (2C); 141.8 (2C); 154.4 (2C); 198.1 (2C) (C quat); Anal Calcld for: $C_{36}H_{34}N_4O_2$, 0.25 H_2O: C, 77.32; H, 6.22; N, 10.01; Found: C, 77.37; H, 6.14; N, 10.05.

*5.1.2.27. 3-{[(9-Allyl-4-hydroxy-9H-carbazol-3-ylmethyl)(pyridin-2-ylmethyl)amino]}-9-allyl-9H-carbazol-4-ol (**6c**).* Crystallization from methanol yielded a white solid; yield 22%, mp = 158 °C; IR (KBr): 1633, 1600, 1583, 1488 cm^{-1}; ^1H NMR (δ, DMSO-d_6): 11.59 (s, 2H, OH); 8.82 (d, 1H, J = 4.7 Hz, H-6′); 8.26 (d, 2H, J = 7.7 Hz, H-5); 7.89 (m, 1H, H-4′); 7.48–7.15 (m, 10H, H aromat.); 6.99 (d, 2H, J = 8.1 Hz, H-1); 5.99–5.90 (m, 2H, CH_2CH=CH_2); 5.09–4.95 (m, 8H, CH_2CH=CH_2 and CH_2CH=CH_2); 3.98 (s, 6H, N-CH_2); ^{13}C NMR (δ, DMSO-d_6): 44.7 (2C); 54.9 (2C); 55.4; 116.4 (2C) (CH_2); 100.2; 108.7 (2C); 118.9 (2C); 122.5; 123.0; 124.0 (2C); 124.6 (2C); 128.4; 133.2 (4C); 138.1; 148.0 (CH); 111.1; 111.6 (2C); 121.8 (2C); 139.4; 139.5; 141.5; 153.1; 156.4 (2C); 159.6; 185.0 (C quat); Anal Calcld for: $C_{38}H_{34}N_4O_2$, 0.5 H_2O: C, 77.66; H, 6.00; N, 9.53; Found: C, 77.52; H, 6.04; N, 9.55.

*5.1.2.28. 3-{[(9-Prenyl-4-hydroxy-9H-carbazol-3-ylmethyl)(pyridin-2-ylmethyl)amino]}-9-prenyl-9H-carbazol-4-ol (**6d**).* Crystallization from methanol yielded a white solid; yield 13%, mp = 148 °C; IR (KBr): 2912, 1635, 1599, 1584 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 11.62 (s, 2H, OH); 8.83 (d, 1H, J = 4.0 Hz, H-6′); 8.51 (d, 2H, J = 7.9 Hz, H-5); 7.73 (m, 1H, H-4′); 7.43–7.13 (m, 10H, H aromat.); 6.87 (d, 2H, J = 8.3 Hz, H-1); 5.30 (t, 2H, J = 5.5 Hz CH_2CH=$C(CH_3)_2$); 4.85 (d, 4H, J = 5.5 Hz, CH_2CH=$C(CH_3)_2$); 4.05 (s, 6H, N–CH_2); 1.93 (s, 6H, CH_3); 1.71 (s, 6H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 18.3 (2C); 25.7 (2C) (CH_3); 41.3 (3C); 55.8 (2C) (CH_2); 99.6 (2C); 107.9 (2C); 109.8; 118.9 (2C); 119.5; 120.2 (2C); 122.7; 123.5; 124.4

(2C); 124.5; 128.2; 137.7; 147.9 (CH); 111.2 (2C); 112.3; 123.6 (2C); 133.2; 134.9 (2C); 139.9 (2C); 142.1 (2C); 152.5; 154.4 (2C) (C quat); Anal Calcld for: $C_{42}H_{42}N_4O_2$, 0.3 H_2O: C, 78.79; H, 6.71; N, 8.75; Found: C, 78.75; H, 6.67; N, 8.78.

*5.1.3. General procedure for the preparation of oxazino[6,5-b] carbazoles (**7**), oxazino[5,6-a]carbazoles (**8**), and dimer (**9**)*

A solution containing 1.5 mmol of primary amine and 3 mmol of formaldehyde dissolved in 10 mL methanol was stirred for 30 min at 0 °C. A solution containing 1 mmol of hydroxycarbazole-*N*-alkylated dissolved in 10 ml of methanol was added dropwise. The mixture was then stirred at room temperature (24 h—several days). At the end of the reaction, the solvent was evaporated under vacuum and the residue was purified by flash chromatography with the corresponding eluting agent.

*5.1.3.1. 7-Allyl-11-methyl-6,7,8,11-tetrahydro[7,9]oxazino[6,5-b]carbazole (**7a**).* Crystallization from petroleum ether yielded a white solid; yield 31%, mp = 105 °C; IR (KBr): 1636, 1605, 1572, 1497 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.93 (d, 1H, J = 7.7 Hz, H-4); 7.63 (s, 1H, H-5 or H-10); 7.39 (m, 1H, H-2 or H-3); 7.32 (d, 1H, J = 7.9 Hz, H-1); 7.16 (m, 1H, H-2 or H-3); 6.78 (s, 1H, H-5 or H-10); 5.95 (m, 1H, CH_2CH=CH_2); 5.23 (m, 2H, CH_2CH=CH_2); 4.97 (s, 2H, H-8); 4.22 (s, 2H, H-6); 3.76 (s, 3H, CH_3); 3.44 (d, 2H, J = 6.4 Hz, CH_2CH=CH_2); ^{13}C NMR (δ, $CDCl_3$): 29.2 (CH_3); 50.1; 54.4; 82.5; 118.9 (CH_2); 95.3; 108.2; 118.8; 118.9; 119.4; 124.7; 135.2 (CH); 111.7; 116.9; 122.8; 141.2; 141.3; 153.7 (C quat); Anal Calcld for: $C_{18}H_{18}N_2O$, 0.33 H_2O: C, 76.04; H, 6.61; N, 9.85; Found: C, 75.93; H, 6.61; N, 9.76.

*5.1.3.2. 7-Benzyl-11-methyl-6,7,8,11-tetrahydro[7,9]oxazino[6,5-b] carbazole (**7b**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 3/7); yield 25%, mp = 187 °C; IR (KBr): 1633, 1602, 1572, 1494 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.83 (d, 1H, J = 7,5 Hz, H-4); 7.52 (s, 1H, H-5 or H-10); 7.32—7.05 (m, 8H, H aromat.); 6.73 (s, 1H, H-5 or H-10); 4.88 (s, 2H, H-8); 4.09 (s, 2H, H-6); 3.88 (s, 2H, CH_2Ph); 3.67 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.2 (CH_3); 50.3; 55.5; 82.8 (CH_2); 95.5; 108.2; 119.0; 119.1; 119.5; 124.7; 127.5; 128.6 (2C); 129.2 (2C) (CH); 111.8; 117.1; 122.9; 138.4; 141.3; 141.4; 153.4 (C quat); HRMS-EI calculated for $C_{22}H_{20}N_2O$, M^+ = 328.1576, Found, M^+ = 328.1573.

*5.1.3.3. 11-Methyl-7-(4-methylbenzyl)-6,7,8,11-tetrahydro[7,9]oxazino [6,5-b]carbazole (**7c**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 3/7); yield 21%, mp = 151 °C; IR (KBr): 1634, 1603, 1570, 1513 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.92 (d, 1H, J = 7.7 Hz, H-4); 7.61 (s, 1H, H-5 or H-10); 7.39—7.15 (m, 7H, H aromat.); 6.82 (s, 1H, H-5 or H-10); 4.98 (s, 2H, H-8); 3.93 (s, 2H, H-6); 3.87 (s, 2H, CH_2Ar); 3.77 (s, 3H, NCH_3); 2.36 (s, 3H, ArCH_3); ^{13}C NMR (δ, $CDCl_3$): 21.3; 29.3 (CH_3); 50.2; 55.5; 82.8 (CH_2); 95.4; 108.2; 119.0; 119.1; 119.5; 124.7; 129.2 (2C); 129.3 (2C) (CH); 111.8; 117.0; 135.3; 137.1; 139.9; 141.3; 144.8; 153.4 (C quat); HRMS-CI calculated for $C_{23}H_{23}N_2O$, MH^+ = 343.1810, Found, MH^+ = 343.1814.

*5.1.3.4. 11-Methyl-7-(α-methylbenzyl)-6,7,8,11-tetrahydro[7,9]oxazino[6,5-b]carbazole (**7d**).* Crystallization from methanol yielded a white solid; yield 42%, mp = 176 °C; IR (KBr): 1636, 1605, 1572, 1471 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.90 (d, 1H, J = 7.7 Hz, H-4); 7.55 (s, 1H, H-5 or H-10); 7.39—7.14 (m, 8H, H aromat.); 6.80 (s, 1H, H-5 or H-10); 5.18 (d, 1H, J = 10.4 Hz, H-8); 4.96 (d, 1H, J = 10.4 Hz, H-8); 4.34 (d, 1H, J = 16.6 Hz, H-6); 4.04 (q, 1H, J = 6.6 Hz, $CH(CH_3)$Ph); 3.99 (d, 1H, J = 16.6 Hz, H-6); 2.17 (s, 3H, NCH_3); 1.50 (d, 3H, J = 6.6 Hz, CH(CH_3)Ph); ^{13}C NMR (δ, $CDCl_3$): 21.7; 29.2 (CH_3); 47.5; 80.6 (CH_2); 57.4; 95.3; 108.2; 118.9 (2C); 119.4; 124.6; 127.3; 127.5 (2C); 128.6 (2C) (CH); 108.2; 112.2; 123.0; 127.3; 141.3; 144.8; 154.1 (C quat); Anal Calcld for: $C_{23}H_{22}N_2O$, 0.2 H_2O: C, 79.83; H, 6.52; N, 8.09; Found: C, 79.65; H, 6.42; N, 8.08.

*5.1.3.5. 11-Methyl-7-pyridin-2-ylmethyl-6,7,8,11-tetrahydro[7,9]oxazino[6,5-b]carbazole (**7e**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/MeOH (5%)); yield 28%, mp = 145 °C; IR (KBr): 1634, 1602, 1588, 1569 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.63 (d, 1H, J = 4.9 Hz, H-6′); 7.92 (d, 1H, J = 7.7 Hz, H-4); 7.69 (m, 1H, H-4′); 7.62 (s, 1H, H-5 or H-10); 7.42—7.14 (m, 5H, H aromat.); 6.83 (s, 1H, H-5 or H-10); 5.03 (s, 2H, H-8); 4.24 (s, 2H, H-6); 4.13 (s, 2H, CH_2Py); 3.78 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.4 (CH_3); 50.6; 57.2; 83.1 (CH_2); 95.5; 108.2; 118.8; 119.0; 119.4; 122.3; 123.2; 124.6; 136.6; 149.8 (CH); 111.5; 117.0; 122.8; 141.2; 141.3; 153.2; 158.4 (C quat); Anal Calcld for: $C_{21}H_{19}N_3O$, 0.25 H_2O: C, 75.54; H, 5.89; N, 12.58; Found: C, 75.50; H, 6.08; N, 12.52.

*5.1.3.6. 11-Methyl-7-phenyl-6,7,8,11-tetrahydro[7,9]oxazino[6,5-b] carbazole (**7f**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 3/7); yield 24%, mp = 182 °C; IR (KBr): 1636, 1598, 1576, 1497 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.94 (d, 1H, J = 7.5 Hz, H-4); 7.71 (s, 1H, H-5 or H-10); 7.42—6.89 (m, 8H, H aromat.); 6.79 (s, 1H, H-5 or H-10); 5.46 (s, 2H, H-8); 4.85 (s, 2H, H-6); 3.73 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.1 (CH_3); 50.9; 80.1 (CH_2); 95.9; 108.2; 118.1; 118.5 (2C); 119.0; 119.5; 121.5; 124.8; 129.4 (2C) (CH); 112.7; 117.2; 117.7; 122.8; 141.4; 148.6; 153.5 (C quat); HRMS-EI calculated for $C_{21}H_{18}N_2O$, M^+ = 314.1419, Found, M^+ = 314.1423.

*5.1.3.7. 7-Benzothiazol-2-ylmethyl-11-methyl-6,7,8,11-tetrahydro[9,7] oxazino[6,5-b]carbazole (**7g**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 1/1); yield 31%, mp = 206 °C; IR (KBr): 1635, 1603, 1575, 1527 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.99 (d, 1H, J = 8.3 Hz, H-4); 7.91 (d, 2H, J = 7.9 Hz, H-4′ et H-7′); 7.62 (s, 1H, H-5 or H-10); 7.50—7.15 (m, 5H, H aromat.); 6.84 (s, 1H, H-5 or H-10); 5.08 (s, 2H, H-8); 4.42 (s, 2H, H-6); 4.34 (s, 2H, CH_2Ar); 3.78 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.2 (CH_3); 51.2; 54.3; 83.1 (CH_2); 95.7; 108.3; 119.0; 119.2; 119.5; 121.9; 123.1; 124.9; 125.1; 126.1 (CH); 111.1; 117.4; 122.8; 135.4; 141.4; 141.5; 152.9; 153.6; 171.9 (C quat); Anal Calcld for: $C_{23}H_{19}N_3OS$, 0.55 H_2O: C, 69.88; H, 5.12; N, 10.63; Found: C, 69.95; H, 5.15; N, 10.20.

*5.1.3.8. 2-Allyl-11-methyl-1,2,3,11-tetrahydro[2,4]oxazino[5,6-a]carbazole (**8a**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 3/7); yield 61%, mp = 99 °C; IR (KBr): 1620, 1595, 1578, 1483 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.95 (d, 1H, J = 7.7 Hz, H-7); 7.83 (d, 1H, J = 8.5 Hz, H-5 or H-6); 7.39 (m, 1H, H-8 or H-9); 7.30 (d, 1H, J = 8.3 Hz, H-10); 7.21 (m, 1H, H-8 or H-9); 6.78 (d, 1H, J = 8.5 Hz, H-5 or H-6); 5.96 (m, 1H, CH_2CH=CH_2); 5.24 (m, 2H, CH_2CH=CH_2); 4.93 (s, 2H, H-3); 4.62 (s, 2H, H-1); 3.94 (s, 3H, CH_3); 3.46 (d, 2H, J = 6.4 Hz, CH_2CH=CH_2); ^{13}C NMR (δ, $CDCl_3$): 32.6 (CH_3); 47.6; 54.7; 81.3; 118.6 (CH_2); 108.4; 109.8; 119.1; 119.3; 119.4; 124.4; 135.1 (CH); 103.0; 117.3; 123.2; 139.5; 141.5; 153.2 (C quat); HRMS-EI calculated for $C_{18}H_{18}N_2O$, M^+ = 278.1419; Found, M^+ = 278.1414.

*5.1.3.9. 2-Benzyl-11-methyl-1,2,3,11-tetrahydro[2,4]oxazino[5,6-a] carbazole (**8b**).* Crystallization from acetone yielded a white solid; yield 51%, mp = 130 °C; IR (KBr): 1636, 1605, 1572, 1497 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.98 (d, 1H, J = 7.5 Hz, H-7); 7.87 (d, 1H, J = 8.5 Hz, H-5 or H-6); 7.43—7.18 (m, 8H, H aromat.); 6.80 (d, 1H, J = 8.5 Hz, H-5 or H-6); 4.97 (s, 2H, H-3); 4.60 (s, 2H, H-1); 4.01 (s, 2H, CH_2Ph); 3.82 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 32.5 (CH_3); 47.9; 55.7; 81.5 (CH_2); 108.4; 109.9; 119.1; 119.3; 119.5; 124.4; 127.6; 128.6 (2C); 129.2 (2C) (CH); 103.1; 117.4; 123.3; 138.3; 139.7; 141.6; 153.3 (C

quat); HRMS-CI calculated for $C_{22}H_{21}N_2O$, $MH^+ = 329.1654$, Found, $MH^+ = 329.1655$.

*5.1.3.10. 11-Methyl-2-(4-methylbenzyl)-1,2,3,11-tetrahydro[2,4]oxazino[5,6-a]carbazole (**8c**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = Ethyl acetate /Petroleum ether: 3/7); yield 42%, mp = 116 °C; IR (KBr): 1619, 1594, 1468, 1443 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.01 (d, 1H, $J = 7.7$ Hz, H-7); 7.90 (d, 1H, $J = 8.5$ Hz, H-6); 7.46–6.88 (m, 7H, H aromat.); 6.82 (d, 1H, $J = 8.5$ Hz, H-5); 4.99 (s, 2H, H-3); 4.64 (s, 2H, H-1); 4.00 (s, 2H, CH_2Ar); 3.90 (s, 3H, NCH_3); 2.42 (s, 3H, ArCH_3); ^{13}C NMR (δ, $CDCl_3$): 21.23; 32.7 (CH_3); 47.8; 55.4; 82.5 (CH_2); 108.5; 109.9; 119.1; 119.3; 119.5; 124.4; 129.2 (2C); 129.3 (2C) (CH); 103.1; 117.4; 122.9; 135.2; 135.3; 137.2; 139.7; 153.3 (C quat); Anal Calcld for: $C_{23}H_{22}N_2O$: C, 80.67; H, 6.48; N, 8.18; Found: C, 80.92; H, 6.68; N, 8.16.

*5.1.3.11. 11-Methyl-2-(α-methylbenzyl)-1,2,3,11-tetrahydro[2,4]oxazino[5,6-a]carbazole (**8d**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/MeOH 2%); yield 21%, mp = 118 °C; IR (KBr): 1621, 1593, 1468, 1443 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 7.95 (d, 1H, $J = 7.7$ Hz, H-7); 7.83 (d, 1H, $J = 8.5$ Hz, H-5 or H-6); 7.40–7.17 (m, 8H, H aromat); 6.75 (d, 1H, $J = 8.5$ Hz, H-5 or H-6); 5.18 (d, 1H, $J = 10.2$ Hz, H-3); 4.97 (d, 1H, $J = 10.2$ Hz, H-3); 4.67 (d, 1H, $J = 16.2$ Hz, H-1); 4.42 (d, 1H, $J = 16.2$ Hz, H-1); 4.24 (q, 1H, $J = 6.6$ Hz, $CH(CH_3)$Ph); 3.71 (s, 3H, NCH_3); 1.53 (d, 3H, $J = 6.6$ Hz, CH(CH_3)Ph); ^{13}C NMR (δ, $CDCl_3$): 21.5; 32.6 (CH_3); 47.7; 79.2 (CH_2); 57.5; 108.3; 109.7; 119.1; 119.2; 119.3; 124.3; 127.4 (3C); 128.6 (2C) (CH); 103.4; 117.2; 123.2; 139.5; 141.5; 144.6; 153.9 (C quat); Anal Calcld for: $C_{23}H_{22}N_2O$, 0.3 H_2O: C, 79.42; H, 6.55; N, 8.05; Found: C, 79.75; H, 6.72; N, 7.73.

*5.1.3.12. 11-Methyl-2-pyridin-2-ylmethyl-1,2,3,11-tetrahydro[2,4]oxazino[5,6-a]carbazole (**8e**).* Purified by column chromatography on silica gel yielded a white solid (Eluent = CH_2Cl_2/MeOH 5%); yield 32%, mp = 131 °C; IR (KBr): 1634, 1602, 1588, 1569 cm^{-1}, ^1H NMR (δ, $CDCl_3$): 8.53 (d, 1H, $J = 4.9$ Hz, H-6′); 7.83 (d, 1H, $J = 7.7$ Hz, H-7); 7.72 (d, 1H, $J = 8.5$ Hz, H-5 or H-6); 7.57 (m, 1H, H-4′); 7.30–7.09 (m, 5H, H aromat.); 6.66 (d, 1H, $J = 8,5$ Hz, H-5 or H-6); 4.86 (s, 2H, H-3); 4.53 (s, 2H, H-1); 4.03 (s, 2H, CH_2Py); 3.70 (s, 3H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 32.3 (CH_3); 48.3; 57.3; 81.7 (CH_2); 108.3; 109.8; 118.9; 119.2; 119.3; 122.3; 123.2; 124.2; 136.6; 149.7 (CH); 102.9; 117.2; 123.1; 139.4; 141.4; 153.1; 158.2 (C quat); HRMS-EI calculated for $C_{21}H_{19}N_3O$, $M^+ = 329.1528$; Found, $M^+ = 329.1523$.

*5.1.3.13. 3-{[(2-Hydroxy-9-methyl-9H-carbazol-3-ylmethyl)(pyridin-2-ylmethyl)amino]}-9-methyl-9H-carbazol-2-ol (**9**).* Crystallization from methanol yielded a white solid; yield 13%, mp = 216 °C; IR (KBr): 1622, 1593, 1571 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 11.04 (s, 2H, OH); 8.72 (d, 1H, $J = 4.7$ Hz, H-6′); 7.94 (d, 2H, $J = 7.5$ Hz, H-5); 7.79 (s, 2H, H-1 or H-4); 7.70 (m, 1H, H-4′); 7.39–7.12 (m, 8H, H aromat.); 6.92 (s, 2H, H-1 or H-4); 4.09 (s, 4H, NCH_2); 4.02 (s, 2H, NCH_2Py); 3.74 (s, 6H, CH_3); ^{13}C NMR (δ, $CDCl_3$): 29.2 (2C) (CH_3); 50.3; 55.5; 82.8 (CH_2); 95.5 (2C); 108.2 (2C); 119.0; 119.1 (2C); 119.5; 124.7; 127.5; 128.6 (3C); 129.2 (3C) (CH); 111.8 (2C); 117.1 (2C); 123.0 (2C); 138.4; 141.3 (2C); 141.4 (2C); 153.4 (2C) (C quat); HRMS-CI calculated for $C_{34}H_{32}N_4O_2$, $MH^+ = 527.2447$, Found, $MH^+ = 527.2451$.

5.2. Biological activity

5.2.1. Cell cultures and antiproliferative assays

The CEM (T-cell leukemia, at 0.2×10^6 cells/mL), Jurkat (acute T-cell leukemia, at 0.6×10^6 cells/mL), Raji (Burkitt's lymphoma, at 0.4×10^6 cells/mL), MCF-7 (breast cancer cell, at 0.2×10^6 cells/mL) and Caco-2 (colorectal cancer, at 0.2×10^6 cells/mL) cell lines, were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum, 1% of non-essential amino acids, 1% of sodium pyruvate, 1% of L-glutamine, 0.1% of gentamicin. The cells densities were selected in order to keep the cells in an exponential phase of growth (viability > 9 by trypan blue exclusion) and to obtain a linear relation between absorbance and cell number. Cells were added just before the test in a 96 well plate, and incubated with the compounds for 72 hours at 37 °C.

Stock solutions of oxazinocarbazoles (25 mM) were prepared in DMSO and stored at −20 °C.

Cells cytotoxicity was measured using the WST-1 (Chemicon® and Takara®) tetrazolium assay following the manufacturer's instructions [31,32]. Ten microliters were added to the wells and incubated for 4 h at 37 °C and the plate was analysed with o an Anthos 2020 absorption photometer (Anthos Labtec Instruments GmbH) at 492 nm. Results were expressed as % of proliferation according to the optical density of treated cells with respect to the optical density of untreated controls. Control cells were cultured with the corresponding concentration of DMSO (0.1%) and no cytotoxic activity was observed. Etoposide (VP-16) was used as positive control.

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