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Antimicrobial and anticancer activities of diazenyl compounds

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ABSTRACT

Three heterocyclic diazenyl compounds were synthesized by diazotization of 3-aminopyridine and aniline, followed by coupling with 8-aminoquinoleine, morpholine, 1,2,3,4-tetrahydroquinoleine, respectively. The obtained azo compounds are brown to yellow in color and were characterized by various methods such as NMR and X-rays. The synthesized compounds were evaluated for their antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans*. The MIC of the tested compounds, determined by the microdilution method, showed potential antimicrobial activity against all tested microorganisms. 1-(3-pyridyl)-2-(morpholin-4-yl)diazene (**8**) and 1-(3-pyridyl)-2-(tetrahydroquinolin-1-yl)diazene (**10**) showed strong activities against *C. albicans* with MIC values of 150 μM and 120 μM respectively. 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl)diazene (**4**) showed the broadest spectrum of activity with good MICs of 1380–2760 μM on the four strains. These diazenyl compounds were also screened for their anticancer activity against breast (MCF-7), lung (Calu-3), pancreas (PANC-1) and prostate (PC-3) cancer cells and on normal fibroblasts. The results showed good anticancer activities on tumor lines with IC_{50} values ranging from 9.4 to 98 μM. The best anticancer activity was obtained with compound **4** on Calu-3 cancer cells with an IC_{50} of 9.4 μM. The results suggest that compound **4** is an interesting scaffold for pharmacomodulation as suggested by ADME study.

Introduction

Numerous antibiotics, including doxorubicin, bleomycin or actinomycin, have been described to promote cancer cell apoptosis or to inhibit cancer cell growth, such preventing tumor metastasis [1,2]. On the other hand, the misuse of antibiotics in recent decades has led to antibiotic resistance, which is now a serious for public health problem affecting all people around the world [3]. Thus, there is an urgent need for the development of new small molecules but in a more simple, effective, and environmentally friendly way [4]. When the azo moiety (—N═N) is coupled to a nitrogen, it leads to a triazene (—N═N—N—), which can be considered as amine-substituted diazenes, while their coupling to a carbon lead to a diazo moiety (—N═N—C). Various diazenyl compounds have been reported to represent molecules with both antimicrobial and antitumoral activities. In our previous work, we have proposed as a perspective to substitute phenyl in diazenyl compound with bioisosters such as quinoline, pyridine, morpholine or piperidine [5]. In fact, various heterocyclic compounds, already used as drug to cure several diseases: tiagabine (antiepileptic) [6], solifenacin (overactive bladder) [7], carmegliptin (Type 2 diabetes) [8], dacarbazine and temozolomide (anticancer) [9], contain piperidine, suggesting that such substitutions could improve the biological activities of diazenyl compounds.

The diazenyl bases have both antimicrobial and cytotoxicity activities [10]. Moreover, a strong therapeutic potential of these compounds has been demonstrated as antioxidant, antimycobacterial, antibacterial,

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and antidiabetic [11] These compounds can be obtained by various synthetic methods, among which the coupling of diazonium salts of aromatic amines with a nucleophilic compound, in aqueous or in organic medium, is one of the most used [12]. Other methods have been reported for the preparation of diazo compounds derivatives, such as the addition of organometallic reagents to alkyl azides [13]. Various routes are also well-known for N1 and N2 substitution by activating the ammonium salt formed from the primary amine. As well, many synthetic methodologies have noticed the C—H directing activation by amide group and various chemical transformations including C—N bonds formation have been developed [14]. For instance, 8-aminoquinoline with its two coordination sites represents an excellent directing group. The modification of the C5 position of 8-aminoquinoline draws much attention in the synthetic strategy [15]. The reaction of C5 of 8-aminoquinoline at room temperature or high temperature (120 °C) [16] can only take place with protection of the amine function. Therefore, functionalization of the 8-aminoquinoline C5—H bond may represent a powerful tool in the synthesis of C5-substituted of various compounds by the formation of a C5-amine bond, as reported in the C—H amination [17], nitration [18], and azidation [19].

Furthermore, diazenes can be obtained synthetically or rarely from natural origin where they can be isolated from microorganisms, plants, fungi, marine organisms, etc., they could prove to be promising candidates for the development of new drugs, such as anticancer, anti-infective, etc. [20]. Jietacins A and B, isolated from a *Streptomyces* species, showed 10-fold higher activities against the pinewood nematode *Bursaphelenchus hgnicolus* compared to avermectin used as a nematocide agent. [20]. Lomaiviticin A with two diazofluorene moieties, related to kinamycins, is extremely cytotoxic with IC_{50} against K562, LNCaP, HCT-116 and HeLa cancer cell lines of 0.12, 0.31, 0.034, 4.5 nM respectively by causing double-stranded DNA breakage. It is more active than kinamycin f, used in cancer treatment, which has IC_{50} of 72, 120, 270 and 520 nM against these same strains, respectively [21].

In this work, we presented for the first time the synthesis, molecular crystalline characterization, and biological activities of three diazo compounds.

Materials and methods

All reagents were obtained from commercial suppliers and used without further purification unless otherwise noted.

Chemistry

Generality

Reactions requiring anhydrous media are performed under an argon atmosphere. All the temperatures of the reactions are indicated. The evolution of the reactions is followed by thin layer chromatography (TLC) using 60 F254 Merck silica gel plates on aluminum support. For purification, pressure column chromatography with silica gel 60 (230–400 mesh) MERCK was used. Melting points are determined with a GALLENKAMP apparatus. NMR spectra are recorded on BRUKER ARX400 (400 MHz) instruments. Chemical shifts (δ) are expressed in ppm relative to a spectral reference ($\delta = 0$ ppm). The solvent signal was used to calibrate the spectrum. (DMSO-d_6 and $CDCl_3$). The mass spectra were recorded by a FISONG VG Autoespec M spectrometer. HPLC chromatograms were recorded on Thermoscientific Dionex ultimate 3000 chromatographs using an UV diode array detector (DAD) and an "ACE 3C18-AR" 150 × 4.6 mm column.

Synthesis of diazo compounds

Several methods are known for the synthesis of diazenes, but the most used is the diazo coupling reaction which begins with formation of diazonium salt by the action of $NaNO_2$ in an acidic medium on a primary amine and coupling of this salt with a primary or secondary amine. In this part of the work, a certain number of difficulties were encountered concerning the solubility of the synthesized diazenes in classical NMR solvents ($CDCl_3$ and aceton-d_6). Known by the strong presence of dipole moment [22], the analysis of these compounds required the use of very polar solvents such as DMSO-d_6.

General process of diazo compounds synthesis

In this general process, we have two main steps: preparation of the diazonium salt and coupling with a primary or secondary amine.

Synthesis in aqueous medium

In a 100 mL flask, para-(trifluoromethyl)aniline (**1**) (10 mmol), concentrated hydrochloric acid (6 N; 6 mL) was introduced. A precooled $NaNO_2$ solution (0.69 g; 10 mmol) was added to the flask under stirring. The mixture is kept stirring for 15 min and at 0 °C (diazonium salt). To another 100 mL flask, 8-aminoquinoleine (**3**) was added and potassium carbonate (2.07 g; 15 mmol) was dissolved in 15 mL of distilled water. The flask is placed in an ice bath (0 °C) and shaken vigorously. Then the solution from the first flask, containing the diazonium salt, was added dropwise to the flask containing the 8-aminoquinoleine (**3**) (coupling). After this addition, the reaction mixture was kept under stirring for 24 h at room temperature. The 1(4-trifluoromethyl)phenyl)-2(8-aminoquinolinyl)diazene **(4)** obtained was extracted with dichloromethane; dried with Na_2SO_4, filtered, and the solvent evaporated (Scheme 1).

Synthesis in organic medium

We have tried several methods for synthesizing diazonium salts of aminopyridine derivatives without success. Finally, we successfully obtained the diazonium salt of 3-aminopyridine using isoamyl nitrile instead of sodium nitrite and ethanol as solvent with good yield. After isolating the diazonium salt by filtration, we witnessed an explosion of our sample because of its supposed instability when exposed to air. To avoid the rapid decomposition of the diazonium salt, we generated it *in situ* from 3-amino pyridine (**5**) and reacted it with morpholine (**7**) or tetrahydroquinoline (**9**) to access the desired triazenes **8** and **10** (Scheme 2).

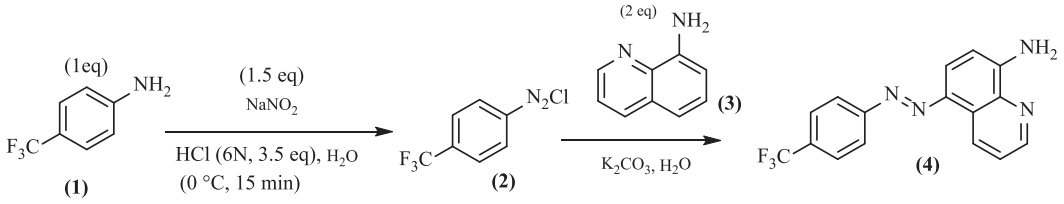
In a 100 mL flask, 3-amino pyridine (5 mmol), ethanol (3 mL), HBF_4 acid (50 % 1.5 mL) and isoamyl nitrite were added. The mixture was kept stirring for 15 min and at −5 °C. Morpholine (**7**) or tetrahydroquinoline (**9**) was added to this solution containing the diazonium salt and then the mixture is stirred for 1 h at 0 °C. Potassium carbonate solution was added to the flask. After this addition, the reaction mixture was kept stirring for 3 h at room temperature. The resulting product **8** or **10** was extracted with ethyl acetate, dried with Na_2SO_4, filtered, and the solvent evaporated.

Biology

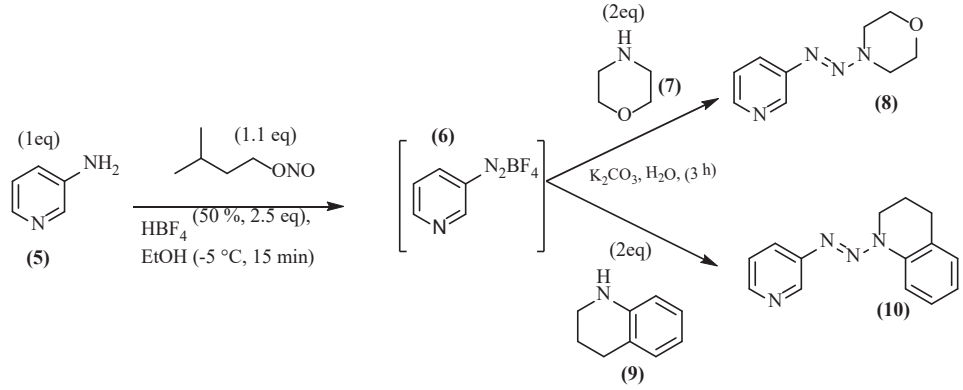
Antimicrobial test

The antimicrobial activity of the synthesized compounds was studied *in vitro* using five reference bacterial strains such as two Gram (+): *E. faec*alis ATCC 29212 and *S. aureus* ATCC 38213; two Gram (−): *E. coli* ATCC 25921 and *P. aeruginosa* ATCC 27253; and one yeast *C. albicans* ATCC 24433. Erythromycin and tetracycline were used as reference compounds.

Inoculum preparation. The microorganisms were first inoculated into the peptone medium and incubated at 37 °C for 24 h. After 24 h of incubation, all microbial strains had grown in their respective media. From the cultures obtained, a microbial suspension in salt solution was made until the inoculum was estimated at 1.5×10^8 CFU/mL based on a turbidity of 0.5 McFarland. Turbidity was measured using a densitometer.



Scheme 1. Access to compound **4.**



Scheme 2. Access to compounds **8** and **10.**

Determination of minimum inhibitory concentrations. The minimum inhibitory concentration was defined as the lowest concentration of the compound that completely suppressed the growth of microorganisms [16]. A range of dilution for each compound was carried out to determine the MIC for each strain. Dilutions were started by pipetting 100 µL of compound into the first well of a 96-well plate containing 100 µL of MH broth. Serial dilutions were carried out by cascade half dilutions to obtain a concentration range between 0.015 and 30 mg/mL. Then, 20 µL of microorganism suspension cultures were added into each well. The plates were then incubated at 37 °C for 24 h. The tested microorganisms were exposed to broth without the alkaloid extract as a control.

Anticancer activity

Cell viability was estimated using a colorimetric assay developed by Mosmann [23]. This method relies on the reduction by living cells of the tetrazolium salt, MTT, to form a blue formazan product [24].

Cell lines. Anticancer activities were determined against breast (MCF-7), lung (Calu-3), pancreas (PANC-1) and prostate (PC-3) cancer cells. All cell lines were purchased from ATCC (American Type Culture Collection/LGC Promochem, Molsheim, France). Telomerase-immortalized corneal fibroblasts (HTK) were used as normal cells [25]. Cells were grown and maintained in DMEM medium complemented with 10 % serum and antibiotics in a humidified incubator at 37 °C with 5 % CO_2.

MTT assay protocol. After trypsinization and counting, cells were seeded at 10 000 cells per well in a 96-well plate. 24h latter, the compounds were added at different concentrations ranging from 0.0005 to 10 µg/mL in sextuplicate and incubated for 72 h at 37 °C. DMSO was used as a negative control and carboplatin as a positive control [24]. MTT (0.5 mg/mL) was then added, and the plate was incubated for an additional hour. After washing, intracellular formazan crystals were dissolved in 100 µL of DMSO and absorbance was measured at 570 nm using a Multiskan® FC plate reader (Thermo Scientific). Cell viability was then estimated in percentage of control cells treated with DMSO alone. Results are expressed as mean ± standard deviation (SD). Chi-square Test were performed using GraphPad Prism to determine differences. P value < 0.05 was considered as statistically significant. IC_{50} was determined using the Quest Graph™ IC_{50} Calculator software [26].

Results and discussion

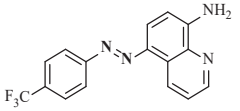
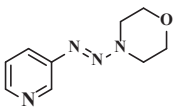
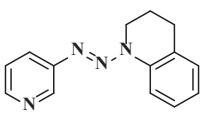
Chemistry

The diazo crystalline compounds (Table 1.) were prepared by treating the appropriate anilines in an aqueous medium (compound **4**) or in an organic medium (compounds **8** and **10**) according to Schemes 1 and 2 respectively. The characterization of the structures is given in the experimental part.

Synthesis

All synthesized compounds gave yields ranging from 30 % to 78 %. Two diazo coupling reactions were observed as expected, leading to compounds **8** and **10**, but also an aromatic electrophilic substitution reaction leading to compound **4**. Indeed, the synthesis in aqueous media led to an unexpected compound: 1-(4-trifluoromethylphenyl)-2-(8-

Table 1
Diazene structures.

Compounds	Structures	Yields (%)
4		30
8		78
10		30

aminoquinolin-5-yl) diazene (**4**) in crystalline form. This result is due to the reactivity of the 5-position of 8-aminoquinoline activated by nitrogen at position 8. This compound has been reported to be synthetized with a better yield by using 8-amimoquinoline and a variety of aryldiazonium salts in aqueous media [27]. A quick comparison of the ^{1}H NMR spectra of compound 4 with that described by Karakaya allows us to note a slight difference between the two in terms of shifts, coupling constants but also resolution for certain signals for example 8.06 (d, J = 8.5 Hz, 1H, H15), 7.99 (d, J = 8.1 Hz, 2H, H4, H6) for us *vs* 8.14–7.95 (m, 3H) for Karakaya) [27]. However, we specify that compound 4 was obtained with an HPLC purity of 98.30 % and that the DRX analysis allowed us to validate the structure of compound 4. ^{13}C NMR shows almost similar shifts except around 126 ppm where we note differences in shifts and signal resolution. The morpholine derivative compound **8** gave a better yield than the tetrahydroquinoline derivative. This could be explained by the fact that the doublet of morpholine nitrogen is more available than the one of the tetrahydroquinoline nitrogen involved in the delocalization of electrons in this nucleus **10**. In many known reactions on 8-aminoquinoline, the reactivity of the carbon requires first the protection of the amino group by an acetylation reaction. This work shows the possibility of direct reaction of C5 of 8-aminotetrahydroquinoline without requiring any additional protection or deprotection. This result offers a uniquely stereo-economical platform to access 8-aminotetrahydroquinoline derivatives with various *N*-substitutions.

X-ray crystallography

Crystal structure determination of 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl)diazene (compound **4**)

The molecular structure of the compound **4**: $C_{16}H_{11}F_3N_4$ (MW = 316.29 g/mol) is shown in the Fig. 1. This compound **4** was recrystallized from ethanol which resulted in yellow singles crystals. Single Crystal, suitable for single-crystal X-ray diffraction analysis was selected and mounted on a Rigaku FRE+diffractometer. The crystal 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl) diazene (**4**) was kept at 300(2) K during data collection. Compound crystallizes in the centrosymmetric monoclinic space group $P2_1/n$, with the asymmetric unit consisting of one molecule of 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl) diazene. The shell parameters were a = 12.9632(3) Å, b = 4.44640(10) Å, c = 24.9820(7) Å, and β = 98.205(2). The final R_1 was 0.0427 ($I > 2\sigma(I)$) and wR_2 was 0.1311 (all data). X-ray diffraction allowed us to confirm that, unlike the triazene expected by diazo coupling of the amine and the diazonium salt, we indeed had an electrophilic substitution reaction of the diazonium salt on position 5 of the 8-aminoquinoline. The compound obtained is 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl) diazene (**4**) confirmed by its crystal structure (Fig. 1 and Table 2). These XRD data alone are sufficient to confirm the ^{1}H and ^{13}C NMR data of compound 9 which overlap well with those given by Karakaya [27].

The molecule is made up of two fragments: a phenyl and an 8-aminoquinoline linked by a diazene fragment (—N=N—). Indeed, the length of 1.225 Å measured for the N3—N4 bond allowed us to conclude that it is a double bond. The dihedral angle between the rings was 4° (2). The C9/ N2/N1/C10 moiety is approximately planar, with a very slight deviation. In the molecule, the phenyl ring and quinoline ring adopt a *Z*-configuration with respect to the N—N double bond and are almost coplanar with a C9—N3 N4—C10 torsion angle of 179.1 (4).

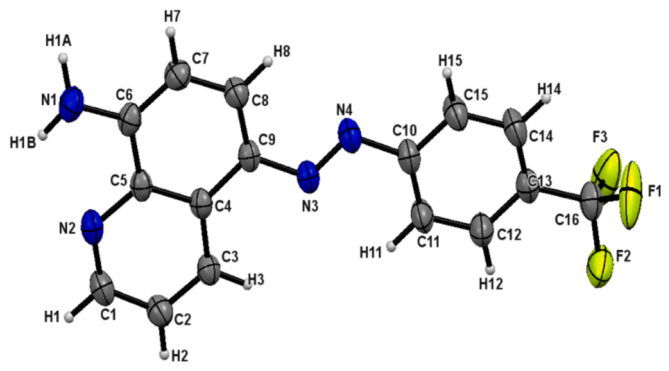


Fig. 1. Crystal structure of 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl) diazene **(4)**.

Table 2
Crystal data and structure refinement for 1-(4-trifluoromethylphenyl)-2-(8-aminoquinolin-5-yl) diazene **(4)**.

Empirical formula	$C_{15.62}H_{11}F_3N_4$
Formula weight	311.75
Crystal system, Space group	Monoclinic, $P2_1/n$
a/Å	12.9632(3)
b/Å	4.44640(10)
c/Å	24.9820(7)
β/°	98.205(2)
Volume/Å^3	1425.21(6)
Z	4
F(000)	639.0
Crystal size/mm^3	0.28 × 0.18 × 0.1
2Θ range for data collection/°	7.15–136.422
Reflections collected	8096
Independent reflections	2563 [R_{int} = 0.0152, R_{sigma} = 0.0182]
Data/restraints/parameters	2563/51/243
Goodness-of-fit on F^2	1.151
Final R indexes [I ≥ 2σ (I)]	R_1 = 0.0600, wR_2 = 0.1892
Final R indexes [all data]	R_1 = 0.0666, wR_2 = 0.1970
Largest diff. peak/hole/e Å^{-3}	0.35/-0.35

In the crystal structure, two molecules are linked via inter-molecular hydrogen bonds type N—H···N and N—H···F into a three-dimensional network.

The determination of the crystal structures of 1-(3-pyridyl)-2-(morpholin-4-yl)diazene (**8**) and 1-(3-pyridyl)-2-(tetrahydroquinolin-1-yl) diazene (**10**) was described in our previous work [28].

Antimicrobial activity

A test for antimicrobial activities of the synthetized compounds were performed. Amikacin, vancomycin, and betadine iodate (10 %) were used as references for antimicrobial activities. The results showed that all controls exhibit antimicrobial activity against all tested strain (data not shown). The minimum inhibitory concentration (MIC) for each synthetized compounds on the same strains were then determined. The obtained results are given in Table 3 and corroborate studies of Silva *et al.* [29] and Paraginski *et al.* [30]. The compounds **8** and **10** exhibit stronger activities against *C. albicans* with MIC values of 150 μM and 120 μM, respectively. *C. albicans*. and *E. faecalis* were found to be the most sensitive strains, regarding the lowest obtained MIC values. As shown in Table 3, compound **4** exhibits the broadest spectrum activity. This could be explained by the presence of the CF_3 function which protects against degradation and increases the metabolic stability of this compound. Indeed, Cappoen *et al.* have also demonstrated that the concentration giving a 90 % reduction of *M. tuberculosis* growth was lower for compounds containing a CF_3 group compared to those lacking it [3a]. Compound **10** showed a similar antimicrobial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* with the same MIC value. We were able to detect activity of the selected compounds both on Gram-positive strains and on Gram-negative strains such as *E. coli;* therefore, we hypothesize that the mode of action of our tested compounds is not membrane penetration.

Anticancer activity

We thus evaluated the anticancer activity of the three diazo compounds using the MTT assay on breast (MCF-7), lung (Calu-3), pancreas (PANC-1) and prostate (PC-3) cancer cells and on normal fibroblasts (HTK). The purpose was to identify compounds that could evidence good anticancer activities and non-cytotoxic ones on normal cells as well

Table 3
Minimal Inhibitory Concentrations MIC (μM) of diazo compounds.

Compounds	Microorganisms				
	E. coli ATCC 25921 G (−)	*P. aeruginosa* ATCC 27253 G (−)	*S. aureus* ATCC 38213 G (+)	*E. faecalis* ATCC 29212 G (+)	*C. albicans* ATCC 24433
4	2760	2760	2760	1380	1380
8	9760	39,040	39,040	2440	150
10	31,490	31,490	31,490	250	120
Erythromycine	NA	2.73	2.73	NA	NA
Tetracycline	NA	9.00	9.00	NA	NA

NA: no activity.

as their selectivity towards cancer cell lines. An initial screening measured the anticancer activity of these three compounds at a concentration of 10 μg/mL. The results obtained are summarized in the Fig. 2.

We could evidence that DMSO has no effect on cell viability. Compound **8** is the less active molecule with cell viabilities ranging from 72 to 100 %. This compound is weakly active on MCF-7 and PANC-1 cancer cells, with viability percentages of 72 and 82 respectively, and is inactive on HTK, Calu-3 and on PC-3 cells. Compound **10** presents an in-between anticancer activity on the Calu-3, PANC-1 and MCF-7 cell lines with viabilities of 44, 58 and 62 %, respectively. Low anticancer activity was also observed with this compound on the PC-3 cell line (75 % viability) and it affects only 27 % of HTK cell viability. Compound **4** presents the best activities on MCF-7 cells with a viability of 42 % and on Calu-3 cells with 31 % viability. However, it is weakly active in PANC-1 cells and has no effect in normal fibroblasts (HTK).

In order to confirm these screening results, the IC_{50} values of the three compounds were determined. Platinum salts were used as a positive control [31]. The results are shown in Table 4. A compound is considered inactive when its IC_{50} value is higher than 250 μM. The IC_{50} values of carboplatin against cancer cells under our experimental conditions are similar to the ones previously described [32]. For the tested compounds, the results show low to good anticancer activities on tumor lines with IC_{50} values ranging from 9.4 to more than 500 μM. The IC_{50} values obtained with compound **8** (>500 μM) confirmed the ineffectiveness of this compound on all tested cancer cell lines. A moderate anticancer activity of compound **10** was found with IC_{50} values of 60; 80 and 98 μM against the PC-3, Calu-3 and PANC-1 cells, respectively. The anticancer activities of this compound **10** are somehow similar to those observed for the platinum salt, excepted on MCF-7 cells. The best anticancer activity, IC_{50} = 9.4 μM), was observed with compound **4** against Calu-3 cells. This compound **4** is also active on PANC-1 cells with an IC_{50} of 62 μM. However, it is inactive against the MCF-7 and PC-3 cell lines (IC_{50} > 250 μM).

Table 4
IC_{50} (μM) of the three diazenyl compounds.

Compounds	Cell lines				
	HTK	MCF-7	Calu-3	PANC-1	PC-3
Carboplatin	187.1	257.5	65*	115.9	50.3
4	>300	>300	9.4	62	>300
8	>500	>500	>500	>500	>500
10	nd	>400	80	98	60

(nd: not determined); (* P value < 0.05).

As part of the study of the structure–activity relationship, El-Senduny describes how the incorporation of the urea functionality into the backbone of organoselenium compounds has made it possible to develop promising chemotherapeutic pathways against liver cancer [31]. In the same context, we were interested in the study of the structure–activity relationship and observed that diazenes substituted on the nitrogen by a bicyclic radical (**4** and **10**) present better activities on cancer cell lines. However, both are inactive against MCF-7 cells. Noticeable selectivity was observed with compound **4** on the Calu-3 cells. These results support the potential of quinoline derivatives already reported against various tumor cell lines [33]. Our results confirm the studies of Kaur *et al.* [10] showing that Schiff diazenyl bases present good cytotoxic activity on the human carcinoma cell line (HCT116) with the compound 4-((2-bromophenyl) diazenyl)-2-((4-nitrophenylimino) methyl) phenol with an IC_{50} value of 17 μM.

A better anticancer activity was obtained with compound **4** on Calu-3 with an IC_{50} value of 9.4 μM, even better than the one for the platinum salt. This prompt us to submit compound **4** to ADME analysis [34]. In terms of physicochemical parameters, this compound presents a low

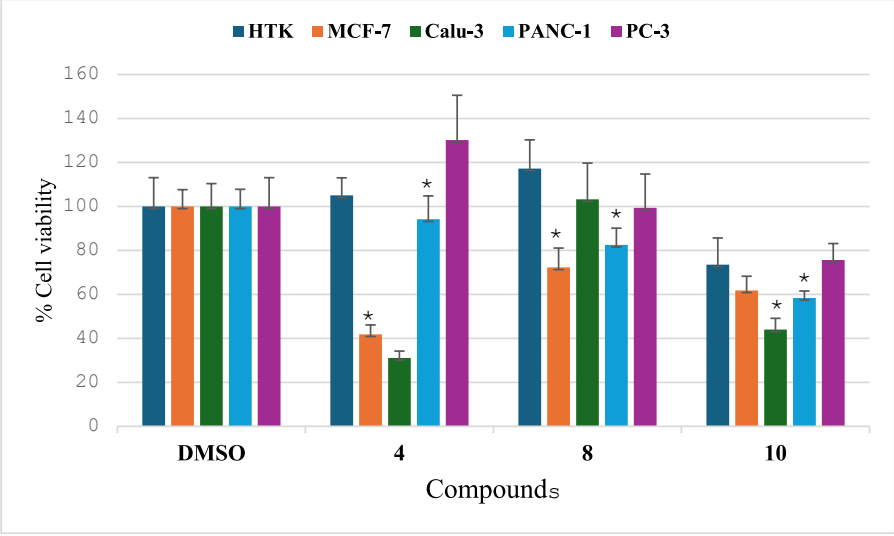


Fig. 2. Screening for anticancer activities of the synthetized compounds (* P value < 0.05).

molecular weight ($MW = 316.29$ g/mol) as well as a correct number of rotatable bonds (3) and a Topological Polar Surface Area of 63.63 Å^2 which is in accordance with its high gastro-intestinal absorption index and its low lipophilicity (consensus Log $P_{o/w} = 4.10$). Compound **4** is moderately soluble in water and had a good skin permeability (Log $K_p = -5.27$ cm/s). Notably, this compound adhered to Lipinski's rule (Ro5) without any violations. In terms of medicinal chemistry, it has only one PAINS (Pan Assay Interference compounds from Screening) alert due to an azo_A function and a synthetic accessibility score of 2.61, thus suggesting unproblematic chemical synthesis. Altogether, these data suggest that compound **4** is a promising scaffold for pharmacomodulation.

The IC_{50} values for compound **10** confirm the moderated activity of arylbicyclo diazenes already reported on other or similar cancer cells [18b]. (E)-1-(4-(3-(benzothiazol-2-yl) triaz-2-en-1-yl)phenyl)ethan-1-one was the most active with an IC_{50} of 130 μM on MCF-7 cells and 34 μM on HCT-116 cells [35]. The non-cytotoxicity of these diazenyl compounds on the normal fibroblasts (HTK cells) is of interest since the major problem in chemotherapy is off finding highly active molecules on cancer cells with few side effects on normal cells.

Conclusion

Three heterocycles diazenyl compounds were synthesized through the diazo coupling method. The diazenyl derivatives were characterized by various spectroscopic techniques. The results of crystallographic studies showed that this pathway for heterocyclic diazenyl and C-N bond formation is possible according to the reactivity of diazonium salts. The tested compounds exhibited different levels of antimicrobial and anticancer activities. Compound **4** containing CF_3 in para position of N1-substituent and free amine showed the broadest spectrum of activity. The best anticancer activity was obtained with this compound **4** on Calu-3 with an IC_{50} of 9.4 μM. Thus, this diazinyl compound, although inactive against normal cells, is very promising in terms of new powerful antimicrobial and anticancer agents and can be further explored for its mechanism of action on microbial strains and cancer cell lines.

CRediT authorship contribution statement

Seynabou Sokhna: Writing – original draft, Resources, Formal analysis, Data curation. **Insa Seck:** Writing – original draft, Supervision, Data curation. **Marc Presset:** Writing – original draft, Validation, Supervision, Methodology, Data curation, Conceptualization. **Eric Huet:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Samba Fama Ndoye:** Writing – original draft, Supervision, Data curation. **Lalla Aïcha Ba:** Writing – original draft, Supervision, Data curation. **Issa Samb:** Writing – original draft, Data curation. **Erwan Le Gall:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization. **Matar Seck:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2024.101749.

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