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Diastereoselective synthesis and cytotoxic evaluation of new isoxazoles and pyrazoles with monoterpenic skeleton

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ABSTRACT

New series of chiral isoxazoles and pyrazoles with monoterpenic skeleton, have been efficiently synthesized from naturally occurring (R)-Carvone, using 1,3-dipolar cycloaddition reaction with arylonitrile oxides and diarylnitrilimines. The reaction showed high peri-, and regioselectivity. In the case of diarylnitrilimines, the reaction revealed to be highly diastereoselective. The structure of the newly synthesized adducts were fully established via spectroscopic analysis and X-ray crystallography. A succinct theoretical study was used to explain the diastereoselectivity experimentally observed. All the newly synthesized monoterpenic isoxazole and pyrazole derivatives were evaluated for their cytotoxic activity against human HT1080, MCF-7 and A-549 cancer cells.

1. Introduction

Nowadays, there is no denying that five-membered heterocycles are of great interest in chemical and pharmaceutical industries. Indeed, many bioactive natural product, pharmaceuticals and agrochemicals bear at least one heterocyclic unit. In addition, heterocyclic systems are important building-blocks for new materials possessing interesting physical or biological properties.

The five membered heterocyclic compounds, particularly with pyrazole and isoxazole nuclei, are components of a diverse array of compounds with a broad spectrum of bioactivities such as analgesic [1–3], anti-inflammatory [3–5], antibacterial [3,6,7], antifungal [3,8,9], antitumoral [3,10,11] and antiviral [3,12,13] activities.

Consequently, various methods for the preparation of these interesting heterocyclic rings were reported in the literature [3,14–16]. Among synthetic routes most often employed, 1,3-dipolar cycloaddition reaction of suitable 1,3-dipole with appropriate dipolarophile is the main approach to access these valuable nucleous, because of its simplicity, efficiency and high selectivity [17–21]. As part of our ongoing research project aiming at the preparation of heterocyclic systems with monoterpenic skeleton [22–28], we report in this work the hemisynthesis of new isoxazoles and pyrazoles, through 1,3-dipolar cycloaddition reaction of naturally occurred (R)-Carvone (**1**), with nitrile oxides and nitrilimines respectively.

Both spectroscopic and theoretical studies had been undertaken to account for the peri-, regio- and diastereo-selectivity of these [3 + 2] cycloaddition reactions. The evaluation of the cytotoxic activity of the newly prepared isoxazole and pyrazole derivatives is also reported here.

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2. Results and discussion

(R)-Carvone (**1**) is a ketonic monoterpene containing two unsymmetrical C=C double bonds. A trisubstituted endocyclic one, activated a withdrawing effect of the carbonyl group in alpha position, while the other is a disubstituted exocylic double bond far from the electronic attractive effect of the carbonyl group. Thus, involvement of these two different dipolarophiles in a 1,3-dipolar cycloaddition will result in regio- and peri-selectivity outcomes (Scheme 1).

An additional diastereoselectivity problem will be encountered, as one or two new stereogenic centers will be generated during the cycloaddition reaction.

2.1. Synthesis of isoxazoles

(R)-Carvone (**1**) was treated, with half stoechiometric amounts of arylonitriles (**2a-e**), generated from the corresponding oximes using commercial bleaching agent [29]. Different concentrations of aqueous NaOCl were tested; the best results were obtained with 5% of NaOCl (Table 1).

The reaction was performed at 0 °C, in dichloromethane as solvent to yield the corresponding isoxazoles (**3a-e**) in a high peri- and regioselevtive manner with good yields (**3a**: 78%, **3b**: 74%, **3c**: 80%, **3d**: 60% and **3e**: 73%) (Scheme 2). All isoxazoles (**3a-e**) were isolated as inseparable (*R*,*R*)/(*R*,*S*) diastereoisomeric mixtures. It is worth noting here that when using stoechiometric quantities of **2a-e**, other products were formed leading to a significant decrease of the desired cycloadducts yield. Furthermore, our approach leads to an improvement in yields compared to the methodology developed by Clapp et al. [30] and subsequently adopted by Ilidirissi et al. [31].

All the prepared isoxazoles (**3a-e**) were fully characterized from their HRMS and NMR (1D & 2D) spectral data. Indeed, their HRMS spectra show the corresponding pseudo-molecular ion MNa^{+} at $m/z = 292.1323$, $m/z = 306.1466$, $m/z = 326.0932$, $m/z = 337.1173$ and $m/z = 334.1790$ respectively, consistent with monocycloadduct molecular formula (**3a**: $C_{17}H_{19}NO_2$, **3b**: $C_{18}H_{21}NO_2$, **3c**: $C_{17}H_{18}ClNO_2$, **3d**: $C_{17}H_{18}N_2O_4$, **3e**: $C_{20}H_{25}NO_2$).

Besides phenyl group resonances (δ^1H 7.1–8.4 ppm; δ^{13}C 123–152 ppm), the salient features of their NMR spectra is the persistence of signals (δ^1H 6.75 ppm; δ^{13}C 144.10–144.58 ppm) due to its internal double bond methine group (=C3–H) and the disappearance of resonances (δ^1H 4.81 & 4.76 ppm; δ^{13}C 110.45 ppm), characterizing the methylene group of the external double bond of starting material. These latters are replaced in ^{1}H NMR spectra, by a two shielded and split doublets ($J = 17$ Hz) between 2.97 and 3.28 ppm while in ^{13}C NMR spectra we note a shielded and split signal at (39.19–44.35) ppm ascribed to isoxazole methylene group H_2C4'. These spectral data clearly show that 1,3-dipolar cycloaddition reaction of arylnitrile oxides with *(R)*-Carvone is highly periselective as only the external double bond was reactive. This cycloaddition reaction was revealed to be also highly regioselective as we noticed in the ^{13}C NMR spectra, a quaternary carbon (C5′) resonance between 87.74 and 89.73 ppm, which makes evidence that the direction of the cycloaddition was unique (the oxygen of the dipoles is linked to the more sterically hindered carbon of the external double bond). However, a splitting of H_2C4', CH_3–C5' signals in ^{1}H NMR spectroscopy and most of the signals in ^{13}C NMR spectra shows the presence of a diastereoisomeric mixture for all the isoxazoles (**3a-e**).

Table 1
Effect of NaOCl concentration on the reaction yield of benzonitrile oxide and (R)-Carvone.

Aqueous [NaOCl]	2.5%	5.0%	7.5%	10.0%
3a yield (%)	71.5	78	72.5	70.5%

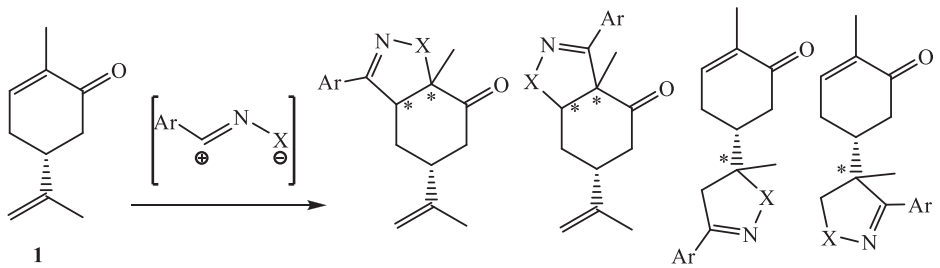
To ascertain all these structural evidences, X-ray analyses were carried out on single crystals of (**3b**) (Fig. 1), which provide noticeable proofs of the assigned structures and allow us to state with certainty that 1,3-dipolar cycloaddition of arylonitrile oxides (**2a-e**) on *(R)*-Carvone (**1**) is highly peri- and regioselective.

The structure is built up by the association of three rings, a cyclohexene, an oxazole and a phenyl ring. The cyclohexene ring has an envelope conformation with puckering parameters $\theta = 126.4°$ and $\varphi = 70.2°$ [32], the oxazole ring is nearly planar with the largest deviation being −0.091(1) Å at C5' but it could be also regarded as having an envelope conformation with the puckering parameter $\varphi = 139.5°$. The dihedral angle between the phenyl and the oxazole rings is 9.3(1)° whereas the dihedral angle between the phenyl and the (C1,C2,C3) and C4 plane is 66.12(8)°.

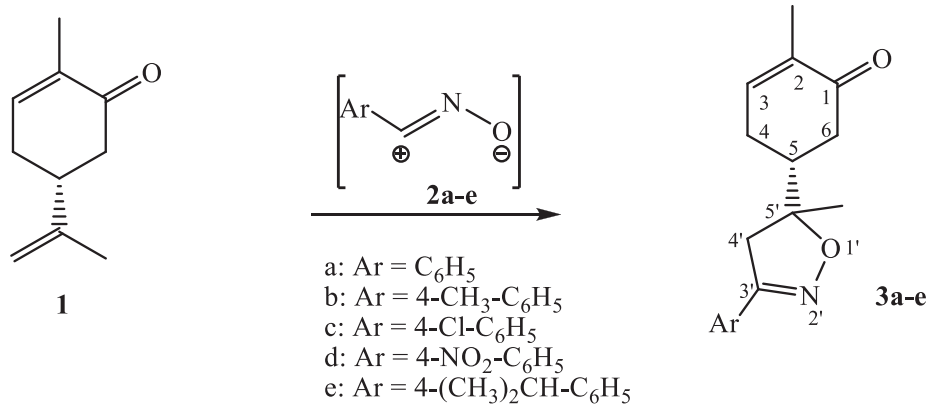
There are (C–H···N) interactions (See Table 2) which stabilize the packing building layer parallel to the (0 0 1) plane. The O1 atom plays acceptor role for four (C–H···O) interactions: C4′-H42, C6–H6′2, C6–H6B, C3–H3 and C5–H5 whereas there is one C–H···N interaction.

2.2. Synthesis of pyrazoles

Aiming at the synthesis of new pyrazoles with monoterpenic skeleton, we have examined the 1,3-dipolar cycloaddition reaction of *(R)*-Carvone (**1**) with stoechiometric quantities of diarylnitrilimines (**4a-d**), generated in situ, from their hydrazonoyl precursors and triethylamine [33]. The reaction was performed, at room temperature, in dichloromethane as solvent to produce the corresponding pyrazoles (**5a-d**) in good yields (**5a**: 84%; **5b**: 84%;



Scheme 1. The two double bonds involved in the reaction.



Scheme 2. The different isoxazoles synthesized in this work.

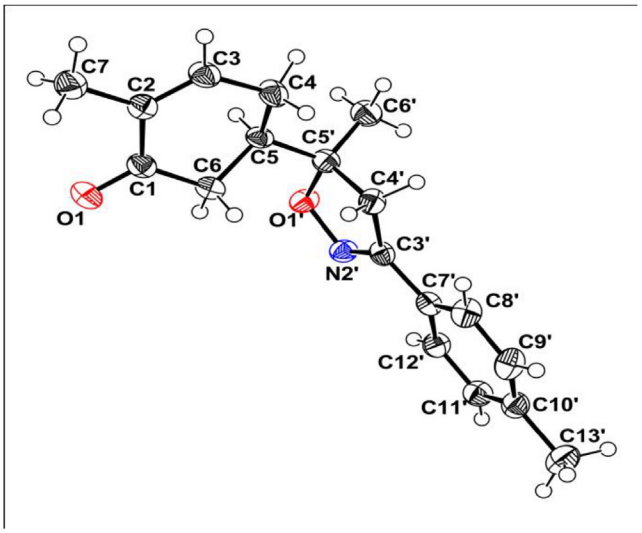


Fig. 1. Molecular view of compound (**3b**) with the atom labeling scheme. Ellipsoids are drawn at the 50% probability level. H atoms are represented as small circle of arbitrary radii.

Table 2
Hydrogen bonds for (**3b**) [Å and °].

D-H … A	d(D-H)	d(H … A)	d(D … A)	<(DHA)
C(4′)-H(4′2) … O(1)^{i}	0.97	2.66	3.615(3)	168.1
C(6′)-H(6′2) … N(2′)^{ii}	0.96	2.64	3.592(3)	172.5
C(6)-H(6B)···O(1)^{i}	0.97	2.57	3.522(3)	167.1
C(3)−H(3) … O(1)^{ii}	0.93	2.60	3.510(3)	167.6
C(5)−H(5) … O(1)^{iii}	0.98	2.66	3.567(3)	153.6

Symmetry code: (i) -x+1,y+1/2,-z+3/2; (ii) x-1,y,z; (iii) -x+1, y-1/2, -z+3/2.

5c: 88%; **5d**: 46%) (Scheme 3).

All pyrazoles (**5a-d**) were isolated as monocycloadducts since they show in their HRMS spectra, pseudo-molecular ion MH^{+} at, $m/z = 345.1972$, $m/z = 359.2116$, $m/z = 379.1569$ and $m/z = 390.1826$ respectively, consistent with the corresponding molecular formula (**5a**: $C_{23}H_{24}N_2O$, **5b**: $C_{24}H_{26}N_2O$, **5c**: $C_{23}H_{23}ClN_2O$, **5d**: $C_{23}H_{23}N_3O_3$). Furthermore, their NMR spectral analysis show evidences that the reaction is highly peri-, regio- and diastereoselective. It should be specified here that (**5a-d**) NMR spectra are closely similar.

A representative example is the pyrazole (**5a**) which mainly reveals in its NMR spectra, signals (two one-hydrogen singlets: δ ^{1}H 4.67 and 4.63 ppm; Csp^2 carbons δ ^{13}C 110.16 and 147.06 ppm) characterizing the external vinylic double bond. Whereas, the internal double bond is not revealed and instead we note C3a (one hydrogen doublet of doublet δ ^{1}H 3.69 ppm J = 1.1 & 6.8 Hz; $HCsp^3$ carbon δ ^{13}C 56.89 ppm) and C7a (δ ^{13}C 73.28 ppm) resonances. This proves that the 1,3-dipolar cycloaddition has occurred solely on the internal double bond.

If well examined, 2D NMR NOESY spectrum of (**5a**) (Fig. 2), shows correlations between C7a-CH_3 methyl group (δ ^{1}H 1.35 ppm) and shielded aromatic hydrogens (two hydrogen doublets $J = 7.9$ Hz at δ ^{1}H 7.12 ppm). This shielding is likely due to the mesomeric donor effect of the sp^3 nitrogen bearing the corresponding phenyl. So, we can clearly state that pyrazole (**5a**) (and the other pyrazoles) is formed as a unique regioisomer where the sp3 nitrogen of the nitrilimine is attached to the more substituted carbon of the C=C internal dipolarophile of the *(R)*-Carvone (**1**).

No split signals were observed in its NMR spectra, therefore, (**5a**), and all the other pyrazoles (**5b-d**) were isolated as single diastereoisomers. However, the absolute configuration of the two newly formed asymmetric carbons C3a and C7a remained unknown. This prompted us to carry out X-ray crystal structural analysis which allowed an unambiguous assignment of the absolute configuration (*R*) to both new stereogenic centers (Fig. 3).

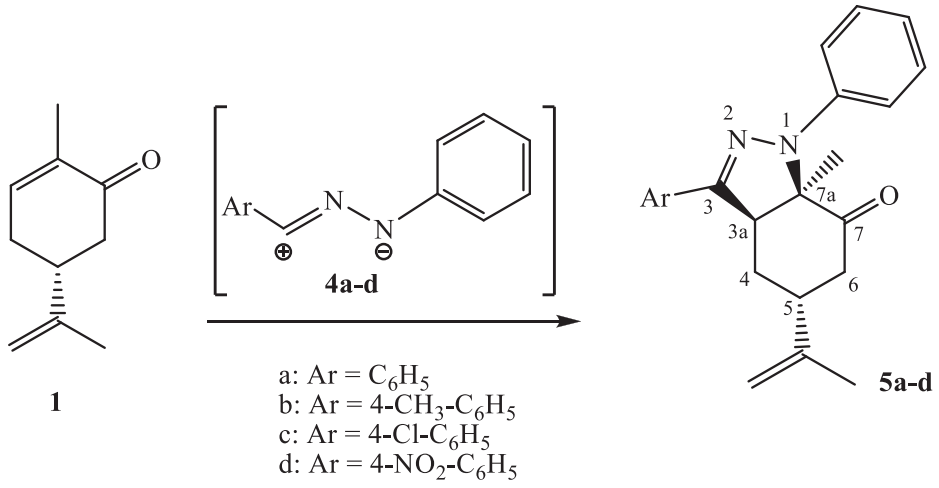
Compound (**5a**) is built up from two fused rings a pyrazole and a cyclohexene. The pyrazole ring is roughly planar with the largest deviation being 0.131(1) Å at C3. However as for compound (**3b**) it could also be considered as having an envelope conformation with the puckering parameter $\varphi = 257°$. The cyclohexene ring has a twist boat conformation as suggested by the puckering parameters: $\theta = 84.8°$ and $\varphi = 275°$. The two phenyl rings attached to the pyrazole display dihedral angles with the pyrazole of 5.3(1)° and 0.7(1)° respectively for the C11 > C16 and C211 > C216 phenyl rings.

There is no (C−H···O) or (C−H···N) intermolecular interactions but there is one (C−H … π) interaction involving C2−H2 and the C211 > C216 phenyl ring (H … Cg^{i} = 2.97 Å, C … Cg^{i} = 3.97 and C−H … Cg^{i} = 174°; symetry code (i) 1-x, −1/2 + y, 3/2-z) (Fig. 4).

Thus, we have been able to prepare efficiently new isoxazoles (**3a-e**) and pyrazoles (**5a-d**), through 1,3-dipolar cycloaddition reaction of *(R)*-Carvone (**1**) with the corresponding 1,3-dipoles. During the synthesis, 1,3-dipolar cycloaddition reaction was revealed to be highly peri- and regio-selective and even diastereoselective in the case of nitrilimines.

3. Computational section

Hoping to explain the diastereoselectivity of the [3 + 2] cycloaddition reaction of (**1**) with, both nitrile oxides (**2a-e**) and



Scheme 3. The different pyrazoles synthesized in this work.

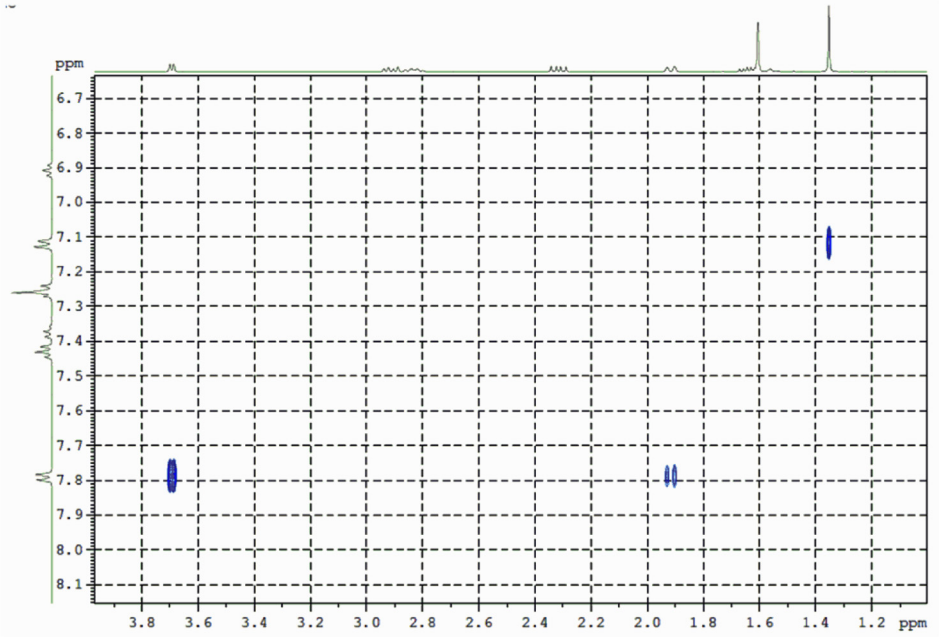


Fig. 2. The NOESY correlations of (**5a**).

diarylnitrilimines (**4a-d**), theoretical calculations were carried out using the density functional theory (DFT) [34]. The structures of the experimentally obtained isoxazoles (**3a-e**) and pyrazoles (**5a-d**) were optimized using the hybrid exchange functional B3LYP [35] and 6-31G* basis set [36] (Fig. 5).

The calculations were performed with the Gaussian 09 set of programs [37]. The investigated structures were fully optimized. Frequencies were calculated to ensure that the optimized structures correspond to the equilibrium geometries of the minima and to obtain the zero-point vibrational energies (ZPE) and thermal correction to enthalpy (TCH). The reported energies including ZPE correction are scaled by the empirical factor 0.9806 [38]. The calculated energies, ZPE and TCH of the relevant species are summarized in Table 1S of the Supporting Information. Enthalpies are calculated as H = E (corrected with ZPE)+TCH. The total and relative energies between *(5R,5′R)* and *(5R,5′S)* in the case of isoxazole derivatives and between (*3aR,5R,7aR*) and (*3aS,5R,7aS*) in the case of pyrazole derivatives are gathered in Table 3.

In terms of ΔE and ΔH, the values of Table 3 show that the stability difference between the (*5R,5′R*) and the (*5R,5′S*) diastereoisomers is relatively weak in the case of isoxazoles (**3a-e**). This result agrees relatively well with the experimental one where we obtain a mixture of the two diastereoisomers. In the case of pyrazoles (**5a-d**), this difference between the (*3aR,5R,7aR*) and the (*3aS,5R,7aS*) diastereoisomers is more accentuated. In term of ΔH it is approximately three times larger than that in the case of isoxazoles (about 9.2 kJ/mol) confirming the experimental result where the (*3aR,5R,7aR*) diastereoisomer was only isolated.

4. Cytotoxic activity evaluation

The synthesized compounds were evaluated for cytotoxic

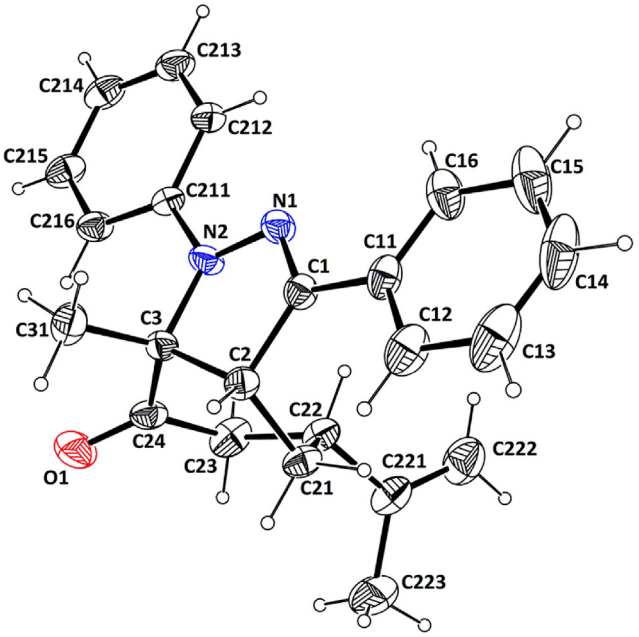


Fig. 3. Molecular view of compound (**5a**) with the atom labeling scheme. Ellipsoids are drawn at the 50% probability level. H atoms are represented as small circle of arbitrary radii.

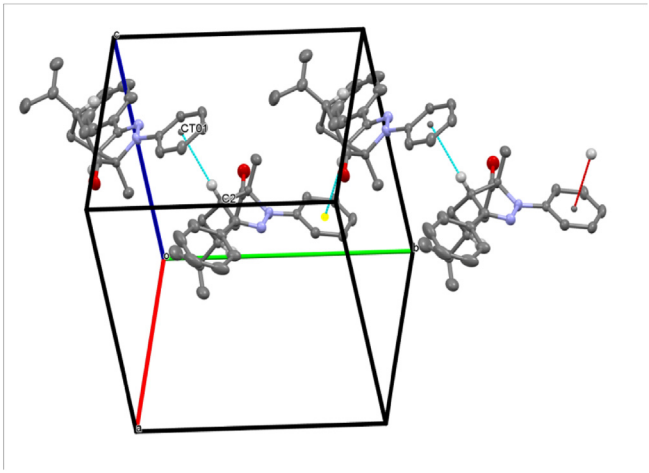


Fig. 4. Partial packing view showing intermolecular C–H … π interaction for compound (**5a**).

activity in vitro against HT-1080, MCF-7 and A-549 cancer cell lines using doxorubicin as a positive control. The IC_{50} are presented in Table 4.

For compounds (**3a),** (**3b**) and (**3d**), the anticancer activities were the highest ones in HT-1080 cells, with IC_{50} values less than 20 μM. However, for the same isoxazoline family the group of molecules including (**3c**) and (**3e**), IC_{50} was over 100 μM. Finally, a last group of pyrazole family compounds (**5a**, **5b**, **5c** and **5d**) has been identified as the less cytotoxic among all molecules with an IC_{50} over 100 μM. In MCF-7 and A-549 cells, several evaluated compounds were more active. In fact, the IC_{50} of the compounds (**3a),** (**3b**), (**3d**), (**3e**) and (**5d**) were the most lower in both cell lines (IC_{50} values less than 30 μM), whereas the cytotoxic activity of compounds (**3c**), (**5a**) and (**5c**) was moderate in A-549 cells. The IC_{50} value of (**5b**) compound was however over 100 μM in these cells. In MCF-7 cells, only the IC_{50} values of **(3c**) and (**5c**) were moderate. Finally, both compounds (5a) and (5b) were the less active in these cells with IC_{50} values over 100 μM.

5. Conclusion

In summary, we have reported an efficient synthesis of new chiral isoxazoles and pyrazoles having monoterpenic skeleton, via 1,3-dipolar cycloaddition reaction of *(R)*-Carvone (**1**) with the corresponding nitrile oxides and nitrilimines. All these pentagonal heterocyclic systems were fully identified using spectroscopic and crystallographic data. The cycloaddition reaction was revealed to be highly peri-, regio- and diastereoselective. This diastereoselectivity was rationalized by a brief computational study. Among monoadducts molecules (**3a-e**) and (**5a-d**), evaluated for their general cytotoxicity against human HT-1080, MCF-7 and A-549 cancer cells, only isoxazoline derivatives (**3a**), (**3b**) and (**3d**) presented an antiproliferative activity in HT-1080 cells. IC_{50} of the compounds (**3a),** (**3b**), (**3d**), (**3e**) and (**5d**) were the most lower in MCF-7 and A-549 cells. The cytotoxic activity of compounds (**3c**), (**5a**) and (**5c**) was moderate in A-549 cells, whereas only the IC_{50} values of **(3c**) and (**5c**) were moderate in MCF-7 cells.

6. Experimental section

All chemicals were used as obtained from commercial sources (Aldrich and Acros). Melting points (m.p.) were determined using a capillary apparatus and are inaccurate. Analytical thin-layer chromatography (TLC) was performed on plates precoated with E. Merck silica gel 60 F254 to a thickness of 0.25 mm. HRMS were obtained on a Q-TOF micromass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with 500 MHz Bruker Avance III spectrometer with a BBFO + probe. Chemical shifts (δ) are expressed in parts per million (ppm). They were recorded relative to solvent $CDCl_3$ signal (7.26 ppm and 77.16 ppm). Arylonitrile oxides (**2a-e**) and diarylnitrilimines (**4a-d**) were generated in situ from the corresponding precursors according to the reported procedures [24,29–33].

Cell culture*:* The human fibrosarcoma cell line HT-1080 (CCL 121) was purchased from Sigma Aldrich (ECACC collection, Saint-Quentin Fallavier, France). The human breast adenocarcinoma MCF-7 (HTB-22) and lung carcinoma A-549 (CCL-185) cell lines were purchased from the American Type Culture Collection (ATCC). Cells were cultured in MEM with Earle salts and Glutamax I (Invitrogen, Cergy-pontoise, France) supplemented with 10% fetal bovine serum, (Invitrogen) and 1% penicillin-streptomycin (Invitrogen). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 (v/v). Cells were routinely passaged at preconfluency using 0.05% trypsin, 0.53 mM EDTA (Invitrogen) and screened for the absence of mycoplasma using PCR methods.

General procedure for the preparation of Isoxazoles (3a-e)*:* To a stirred solution of *(R)*-Carvone (**1**) (6.57 mmol) and p-substituted benzaldoximes (3.28 mmol) in CH_2Cl_2 (15 mL) was added dropwise (during 30min) at 0 °C 20 mL of aqueous NaOCl (5.2%; 15,65 mmol). After 10 min stirring (the reaction was monitored by TLC) at room temperature, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by column chromatography using hexane/Ethylacetate mixture (88:12) as eluent.

6.1. (R)-2-Methyl-5-((R/S)-5-methyl-3-phenyl-4,5-dihydroisoxazol-5-yl)cyclohex-2-enone (3a)

Yield 78%; white solid: mp = 107 °C (Ethanol); HRMS (TOF-MS

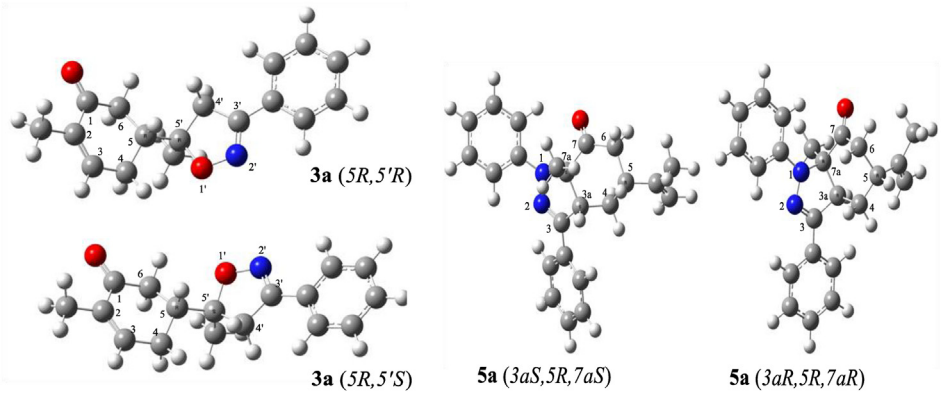


Fig. 5. Optimized structures of the two diastereoisomers of isoxazole **3a** and pyrazole **5a,** at the B3LYP/6-31G* level.

Table 3
B3LYP/6-31G* total (E and H, in au) and relative (ΔE, ΔH, in kJ/mol) energies and enthalpies, of **(*5R,5′R*)** and **(*5R,5′S*)** diastereoisomers in the case of isoxazole derivatives and **(*3aR,5R,7aR*)** and **(*3aS,5R,7aS*)** in the case of pyrazole derivatives.

Isoxazoles						
	E[a]		ΔE	H		ΔH
	(*5R,5′R*)	**(*5R,5′S*)**		**(*5R,5′R*)**	**(*5R,5′S*)**	
3a	−864.081795	−864.080499	−3.40	−864.062872	−864.061571	−3.42
3b	−903.3731931	−903.3718655	−3.48	−903.352378	−903.351045	−3.50
3c	−1323.687004	−1323.685738	−3.32	−1323.66689	−1323.66556	−3.47
3d	−1068.580239	−1068.578974	−3.32	−1068.55879	−1068.55749	−3.42
3e	−1212.906444	−1212.906013	−1.13	−1212.87877	−1212.87852	−0.66
Pyrazoles						
	(*3aR.5R,7aR*)	**(*3aS,5R,7aS*)**		**(*3aR,5R,7aR*)**	**(*3aS,5R,7aS*)**	
5a	−1075.172352	−1075.169115	−8.50	−1075.14901	−1075.14534	−9.64
5b	−1114.46314	−1114.4599	−8.51	−1114.43779	−1114.43416	−9.54
5c	−1534.778142	−1534.775342	−7.36	−1534.75356	−1534.75027	−8.64
5d	−1279.675813	−1279.672859	−7.76	−1279.64996	−1279.64655	−8.94

[a] : Total energies are corrected by ZPE which is scaled by the empirical factor of 0.9806.

Table 4
Cytotoxic activities of the synthesized (**3a-e**) and (**5a-d**) compounds against human HT-1080, MCF-7 and A-549 cells.

Compound	IC50 (μM)		
	HT-1080	MCF-7	A-549
3a	16.10	23.84	27.87
3b	10.72	19.52	15.24
3c	>100	48.24	61.31
3d	9.02	20.24	18.14
3e	>100	22.14	28.01
5a	>100	>100	52.17
5b	>100	>100	>100
5c	>100	49.33	51.66
5d	>100	24.19	27.18

ES+) (m/z): found 292.1323 $[M+H]^+$, calculated 292.1313. ^{1}H NMR δ (ppm): 1.43 &1.47 (3H, 2s); 1.77 (3H, s); 2.36 (1H, m); 2.26−2.33 (2H, m); 2.45−2.64 (2H, m); 3.00−3.30 (2H, 2d J = 16.73 Hz); 6.75 (1H, m); 7.38 (3H, m); 7.62 (2H, m). ^{13}C NMR δ (ppm):15.74 (CH_3); 23.11 & 24.42 (CH_3); 27.13 &27.23 (CH_2); 39.37 & 39.74 (CH_2); 43.91 & 44.16 (CH); 43.95 & 43.37 (CH_2); 88.08 & 88.00 (C5'); 126.56 (HC_{Ar}); 128.84 (HC_{Ar}); 129.77&129.70 (C_{Ar}); 130.18 (HC_{Ar}); 135.60 & 135.54 (=C); 144.43 &144.50 (HC =); 155.74 & 155.89 (C=N); 199.08 & 198.95 ppm (C=O).

6.2. (R)-2-Methyl-5-((R/S)-5-methyl-3-p-tolyl-4,5-dihydroisoxazol-5-yl)cyclohex-2-enone (3b)

Yield 74%; white solid: mp = 96 °C (Ethanol); HRMS (TOF-MS ES+) (m/z): found 306.1466 $[M+H]^+$, calculated 306.1470. ^{1}H NMR δ (ppm): 1.43 & 1.46 (3H, 2s); 1.78 (3H, s); 2.38 (3H, s); 2.52 (1H, m); 2.25−2.37 (2H, m); 2.55−2.63 (2H, m); 2.95−3.30 (2H, 2d J = 16.62 MHz); 6.76 (1H, m); 7.20 (2H, d *J* = 7.98 Hz); 7.53 (2H,dd J = 8.28 & 2.04 Hz). ^{13}C NMR *δ* (ppm):15.76 (CH_3); 21.57 (CH_3); 23.08 & 24.46 (CH_3); 27.16&27.26 (CH_2); 39.42&39.81 (CH_2); 43.48&43.94 (CH); 44.00 & 44.35 (CH_2); 87.77 & 87.83 (C5'); 126.90 & 126.67 (CAr); 126.53 (HCAr); 129.56 (HCAr); 135.55 & 135.61 (=C); 140.45 (CAr); 144.50 & 144.58 (HC =); 155.71 &155.89 (C=N); 199.04 & 199.16 ppm (-C=O). Single crystals of **3b** suitable for X-ray analysis were obtained by slow crystallization from Chloroform solution.

6.3. (R)-2-Methyl-5-((R/S)-5-methyl-3-p-chlorophenyl-4,5-dihydroisoxazol-5-yl)cyclohex-2-enone (3c)

Yield 80%; white solid: mp = 104 °C (Ethanol); HRMS (TOF-MS ES+) (*m/z*): found 326.0932 $[M+H]^+$, calculated 326.0924. ^{1}H NMR *δ* (ppm):1.42 & 1.47 (3H, 2s); 1.77 (3H, s); 2.37 (1H, m); 2.22−2.30 (2H, m); 2.42−2.63 (2H, m); 2.95−3.30 (2H, 2d *J* = 16.74 Hz); 6.75 (1H, m); 7.36 (2H, d *J* = 7.88 Hz); 7.5 (2H,dd J = 8.15 & 1.75 Hz). ^{13}C

NMR δ (ppm): 15.74 (CH_3); 23.07&24.38 (CH_3); 27.11&27.21 (CH_2); 39.31 & 39.71 (CH_2); 43.26 &43.91 (CH_2); 43.98 & 43.88 (CH); 88.40 & 88.50 (C5'); 127.79 (HC_{Ar}); 128.29 (C_{Ar}); 129.12 (HC_{Ar}); 135.59 & 135.65 (=C); 136.11 (C_{Ar}); 144.31 & 144.39 (HC =); 154.82 & 154.97 (C=N); 198.80 & 199.95 ppm (C=O).

6.4. (R)-2-Methyl-5-((R/S)-5-methyl-3-p-nitrorophenyl-4,5-dihydroisoxazol-5-yl)cyclohex-2-enone (3d)

Yield 60%; white solid: mp = 121 °C (Ethanol); HRMS (TOF-MS ES+) (*m*/*z*): found 337.1173 $[M+H]^+$, calculated 337.1164. ^{1}H NMR δ (ppm): 1.46 &1.46 (3H, 2s); 1.77 (3H, s); 2.40 (1H, m); 2.22−2.33 (2H, m); 2.46−2.63 (2H, m); from 3.02 to 3.33 (2H, 2d *J* = 16.75 Hz); 6.75 (1H, m); 7.80 (2H, d *J* = 7.45 Hz); 8.25 (2H,d *J* = 8.80 Hz). ^{13}C NMR δ (ppm):15.71 (CH_3); 23.14 & 24.33 (CH_3); 27.05 & 27.15 (CH_2); 39.19 & 39.57 (CH_2); 43.83 (CH); 42.90 & 43.43 (CH_2); 89.59 & 89.7 (C5'); 124.12 (HC_{Ar}); 127.26 (HC_{Ar}); 135.80 & 135.87 (=C); 135.67 (C_{Ar}); 144.10 & 144.15 (HC =); 148.49 (C_{Ar}); 154.32 (C=N); 198.68 & 198.50 (C=O).

6.5. (R)-2-Methyl-5-((R/S)-5-methyl-3-p-isopropylphenyl-4,5-dihydroisoxazol-5-yl)cyclohex-2-enone (3e)

Yield 73%; white solid: mp = 111 °C (Ethanol); HRMS (TOF-MS ES+) (*m*/*z*): found 334.1790 $[M+H]^+$, calculated 334.1783. ^{1}H NMR δ (ppm):1.22 & 1.27 (6H, 2s); 1.42 &1.46 (3H, 2s); 1.77 (3H, s); 2.30 (1H, m); 2.22−2.34 (2H, m); 2.46−2.63 (2H, m); 2.90 (1H, m); 2.90−3.40 (2H, 2d *J* = 16.75 Hz); 6.75 (1H, m); 7.25 (2H, d *J* = 8.55 Hz); 7.57 (2H,dd J = 8.30 & 1.70 Hz).^{13}C NMR δ (ppm):15.73 (CH_3); 23.10 & 24.40 (CH_3); 23.89 (CH_3); 27.13 & 27.24 (CH_2); 39.39 & 39.57 (CH_2); 34.17 (CH); 43.96 & 44.30 (CH); 43.45 & 43.90 (CH_2); 87.74 & 87.80 (C5'); 126.64 (HC_{Ar}); 126.92 (HC_{Ar}); 127.24 & 127.31 (C_{Ar}); 135.51 & 135.56 (=C); 144.46 & 144.54 (HC =); 151.33 (C_{Ar}); 155.69 (C=N); 199.10 & 198.99 (C=O).

General procedure for the preparation of Pyrazoles (5a-d): To a solution of (*R*)-Carvone (**1**) (0.23 g, 1.37 mmol) and hydrazonoyl chlorides, precursors of diarylnitrilimines (**4a-d**) (1.37 mmol) in dichloromethane (20 mL), triethylamine (0.3 mL) in dichloromethane (2 mL) was added slowly at room temperature. The reaction mixture was then, stirred at room temperature for three days (monitoring by tlc), filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography using hexane/ethyl acetate 96:4.

6.6. (3aR,5R,7aR)-7a-methyl-7-oxo-1,3-diphenyl-5-(prop-1-en-2-yl)-3a,4,5,6,7,7a-hexahydrobenzopyrazole (5a)

Yield 84%; brown solid: mp = 99 °C (Ethanol); HRMS (TOF-MS ES+) (*m*/*z*): found 345.1972 $[M+H]^+$, calculated 345.1967. ^{1}H NMR δ (ppm):1.35 (3H, s); 1.61 (3H, s); 1.65 (1H, m); 1.80−2.50 (2H, m); 2.60−3.00 (2H, m); 3.69 (1H, ddJ = 6.80 & 1.10 Hz); 4.63 (1H, s); 4.68 (1H, s); 6.90 (1H, t *J* = 7.30 Hz); 7.12 (2H, dd J = 8.7 & 1.0 Hz); 7.25 (2H, t *J* = 8.1 Hz); 7.36 (1H, m); 7.42 (2H, m); 7.79 (2H, dd J = 7.15 & 1.5 Hz). ^{13}C NMR δ (ppm):17.62 (CH_3); 20.60 (CH_3); 29.42 (CH_2); 37.77 (CH); 44.20 (CH_2); 56.88 (HC3a); 73.27 (C7a); 110.16 (H_2C =); 114.99 (HC_{Ar}); 120.82 (HC_{Ar}); 126.31 (HC_{Ar}); 128.98 (HC_{Ar}); 129.11 (HC_{Ar}); 129.40 (HC_{Ar}); 131.16 (C_{Ar}); 143.92 (N-C_{Ar}); 147.08 (=C); 149.89 (=C3); 212.22 (C=O). Single crystals of **5a** suitable for X-ray analysis were obtained by slow crystallization from Chloroform solution.

6.7. (3aR,5R,7aR)-7a-methyl-3-p-methylphenyl-7-oxo-1-phenyl-5-(prop-1-en-2-yl)-3a,4,5,6,7,7a-hexahydrobenzopyrazole (5b)

Yield 85%; oil. HRMS (TOF-MS ES+) (*m*/*z*): found 359.2116 [M+H]+, calculated 359.2123. ^{1}H NMR δ (ppm): 1.34 (3H, s); 1.60 (3H, s); 1.64 (1H, m); 1.87−2.35 (2H, m); 2.40 (3H, s); 2.76−3.00 (2H, m); 3.67 (1H, dd J = 6.75 & 1.2 Hz); 4.63 (1H, s); 4.70 (1H, s); 6.90 (1H, t *J* = 7.30 Hz); 7.11 (2H, d *J* = 7.8 Hz); 7.25 (4H, m); 7.68 (2H, d *J* = 8.15 Hz). ^{13}C NMR δ (ppm):17.56 (CH_3); 20.60 (CH_3); 20.60 (CH_3); 29.47 (CH_2); 37.76 (CH); 44.20 (CH_2); 57.00 (HC3a); 73.14 (C7a); 110.11 (H_2C =); 114.95 (HCAr); 120.66 (HCAr); 126.28 (HCAr); 128.33 (CAr); 129.36 (HCAr); 129.68 (HCAr); 139.29 (CAr); 144.04 (N-CAr); 147.08 (=C); 150.06 (=C3); 212.39 (C=O).

6.8. (3aR,5R,7aR)-3-p-chlorophenyl-7a-methyl-7-oxo-1-phenyl-5-(prop-1-en-2-yl)-3a,4,5,6,7,7a-hexahydrobenzopyrazole (5c)

Yield 88%; oil. HRMS (TOF-MS ES+) (*m*/*z*): found 379.1569 $[M+H]^+$, calculated 379.1577. ^{1}H NMR δ (ppm): 1.35 (3H, s); 1.61 (3H, s); 1.65 (1H, m); 1.80−2.40 (2H, m); 2.70−3.00 (2H, m); 3.65 (1H, dd J = 6.70 & 1.25 Hz); 4.60 (1H, s); 4.68 (1H, s); 6.92 (1H, t *J* = 7.30 Hz); 7.12 (2H, d *J* = 8.30 Hz); 7.27 (2H, t *J* = 8.50 Hz); 7.40 (2H, d *J* = 8.70 Hz); 7.72 (2H, d *J* = 8.60 Hz). ^{13}C NMR δ (ppm):17.67 (CH3); 20.56 (CH3); 29.37 (CH2); 37.72 (CH); 44.08 (CH2); 56.71 (HC3a); 73.36 (C7a); 110.25 (H2C =); 114.97 (HCAr); 121.02 (HCAr); 127.44 (HCAr); 129.20 (HCAr); 129.42 (HCAr); 129.64 (CAr); 134.87 (CAr); 143.63 (N-CAr); 146.84 (=C); 148.72 (=C3); 211.78 (-C=O).

6.9. (3aR,5R,7aR)-7a-methyl-3-p-nitrophenyl-7-oxo-1-phenyl-5-(prop-1-en-2-yl)-3a,4,5,6,7,7a-hexahydrobenzopyrazole (5d)

Yield 46%; brown solid mp = 70 °C (Ethanol). HRMS (TOF-MS ES+) (*m*/*z*): found 390.1826 $[M+H]^+$, calculated 390.1818. ^{1}H NMR δ (ppm): 1.40 (3H, s); 1.60 (3H, s); 1.67 (1H, m); 1.80−2.40 (2H, m); 2.70−2.93 (2H, m); 3.71 (1H, dd J = 6.55 & 1.10 Hz); 4.30 (1H, s); 4.69 (1H, s); 6.97 (1H, t *J* = 7.35 Hz); 7.42 (2H, d *J* = 7.85 Hz); 7.28 (2H, dd J = 8.60 & *J* = 7.40 Hz); 7.91 (2H, d *J* = 8.95 Hz); 8.28 (2H, d *J* = 8.95 Hz). ^{13}C NMR δ (ppm):17.99 (CH_3); 20.48 (CH_3); 29.34 (CH_2); 37.70 (CH); 43.95 (CH_2); 56.19 (HC3a); 73.92 (C7a); 110.47 (H_2C =); 115.11 (HC_{Ar}); 121.83 (HC_{Ar}); 124.36 (HC_{Ar}); 126.45 (HC_{Ar}); 129.56 (HC_{Ar}); 137.40 (C_{Ar}); 142.82 (N-C_{Ar}); 146.53 (=C); 147.19 (=C3); 147.41 (C_{Ar}); 210.86 (-C=O).

X-ray structural analyses: Single crystal of each compound was mounted under inert perfluoropolyether at the tip of glass fiber and cooled in the cryostream of either a Rigaku Oxford Diffraction GEMINI EOS diffractometerfor (**3b**) or a Bruker Nonius APEXII for (**5a**). The structures were solved by direct methods SHELXT-2015 [39] and refined by least-squares procedures on F^2 using SHELXL-2014 [40]. All H atoms attached to carbon were introduced in calculation in idealised positions and treated as riding models. Owing to the absence of atoms heavier than Si, the absolute configuration could not be reliably established by refinement of the Flack's parameter [41] but it is confirmed by the synthetic procedure. The drawing of the molecules was realised with the help of ORTEP32 [42]. Crystal data and refinement parameters are shown in Tables (2S−4S).

CCDC 1910555−1910556 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Cytotoxicity Assay: Cytotoxicity of the synthesized compounds was assessed by the CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) (Promega, Charbonnières les Bain, France). Briefly, the cells were plated at a density of 2500 cells/well in a 96-well plate with 100 μL culture medium per well, containing different concentrations of the previously cited molecules (6.25, 12.5, 25, 50 and 100 μM). After 24 h, 15 μL of MTT dye solution was added in each well. The plates were further incubated for 4 h. Then, 100 μL of

the solubilization/stop solution was added into each well and the plate was incubated 1h at room temperature. The optical density of each well was measured at 570 nm using a microplatereader Revelation 96-well multiscanner (Dynex Technologies, Chantilly, VA). Results were represented as a percentage of cell survival with respect to untreated controls. The IC_{50} was defined as the drug concentration required for inhibition of cell growth by 50%, relative to untreated controls. IC_{50} values were estimated from the dose response curve.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2019.126924.

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