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Nitrones and nucleobase-containing spiro-oxindoles derived from isatin and indanone: solvent-free microwave-assisted stereoselective synthesis and theoretical calculations[†]

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The spiro-oxindoles have found wide application because of their antiviral properties. However, in the literature few examples of synthesis of their precursors, oxindole-nitrones, are reported. In this paper, we initially present a rapid and efficient synthetic approach to ketonitrones by solvent-free microwave-assisted reaction between isatin or indanone derivatives and various hydroxylamines. The synthetic protocol is facile, clean, fast, high-yielding and stereoselective. Then, we explored the possibility to synthesize nucleobase-containing spiro-oxindoles with isatin and indanone nuclei by solvent-free MW-assisted 1,3-dipolar cycloaddition, obtaining good results in yields (74–85%), and regio- and diastereoselectivity. Theoretical calculations were done to analyze the difference of reactivity of isatin and indanone derivatives with hydroxylamines.

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Introduction

The spiro-oxindole scaffold is the characteristic structural core of numerous alkaloids and unnatural biologically active compounds.¹ Inspired by this important moiety, a number of methods to synthesize isatin and oxindole derivatives were realized during the last few years. In fact, the biological importance of compounds containing spiro-carbon at the C-3 position of the indoline or oxindole skeleton has recently been emphasized in the literature.^{2,3} In particular, spiro-oxindoles and spiro-oxindoles possess antiviral activity for various human diseases, inhibiting poxvirus,⁴ ectromelia,⁵ rhinovirus,⁶ HIV-1 (ref. 7) and, more recently, as inhibitors of MDM2/p53 interaction.^{8,9}

The 1,3-dipolar cycloaddition between a ketonitrone and a vinylic substrate may be considered a common procedure for

the preparation of these compounds.^{10–12} Although a broad range of methodologies to synthesis of aldonitrones is available today,^{13–20} in contrast the preparation of ketonitrones is not always accomplished by simple condensation reaction.^{21–23} A thorough search of the relevant literature yielded only few articles related to synthetic procedures of isatin and oxindole nitrone.^{24,25} In particular, a methodology regards the preparation of N-substituted isatin nitrones *via* a multi-step reaction sequence.²⁶

Recently, the synthesis of (Z)-N-aryl oxindole nitrones was performed through a *N*-arylation of 3-(hydroxyimino) indolin-2-ones with diaryliodonium salts, demonstrating the current interest in having efficient routes towards those sort of nitrones.²⁷ On the contrary, to our knowledge, methodologies of indanone nitrones are not present in literature.

As a result, the development of a simple and convenient method for the synthesis of isatinyl and indanyl nitrones realized from ketones and hydroxylamines through mild conditions could be highly desirable.

In recent years, the use of microwave technology in organic chemistry, allows to prepare organic compounds very fastly, with high purity and better yield respect to other conventional methods.^{28,29}

Recently, we described a green approach to synthesize aldo- and ketonitrones by solvent-free condensation of alkyl- or aryl-hydroxylamines hydrochlorides with aromatic aldehydes and ketones under microwave irradiation, bypassing the critical results obtained with ketonitrones.³⁰

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Then, in present work, we firstly describe a facile synthesis of isatinyl and indanyl nitrones *via* microwave-assisted reaction between ketones and hydroxylamines under solvent-free conditions. In our approach, we observed very short reaction times and a reduced formation of by-products, isolating the expected products in high yields and excellent stereoselectivity. Successively, we report for the first time a regio- and diastereomeric synthesis of nucleobase-containing spiro-isoxazolidines with an indoline or an indane ring by MW-assisted 1,3-dipolar cycloaddition, obtained in yields between 74–85% and *via* solvent-free procedure.

Results and discussion

To find the optimal conditions for the synthesis of nitrones, we chose *N*-methylhydroxylamine hydrochloride **1a** and 1-isatin **2a** and indanone **3a** as ketones. The methodology consists of the co-grinding of the ketone and hydroxylamine in a mortar, followed by transfer of the mixture in a sealed vessel and further mixing by a vortex; finally the mixture is placed in a microwave oven. The optimization study is collected in Table 1.

The best conditions for both ketones (entries 5 and 11) were those corresponding to an irradiation of 600 W ($T = 180^\circ\text{C}$) for 10 min for ketone **2a** and an irradiation of 400 W ($T = 180^\circ\text{C}$) for 30 min for ketone **3a**. The corresponding products **4a** and **5a** were obtained in 97% and 82% chemical yield, respectively. In both cases, nitrones were obtained as single isomers, *E*-**4a** and *Z*-**5a** respectively, as confirmed by NOESY experiments. When the reaction was carried out in EtOH/H₂O as a solvent and in the

presence of 2 equivalent of sodium acetate as reported in literature on similar substrates,³¹ compounds **4a** and **5a** were obtained in 70% and 78% chemical yield, respectively, in 24 hours (entries 7 and 12). Moreover, the presence of sodium acetate in reaction mixture does not significantly change the trend of the reaction (entry 6) and therefore we chose not to use it.

In an effort to expand the scope of the reaction, we explored coupling of substituted ketones with *N*-methyl hydroxylamine hydrochloride (**1a**) *N*-benzyl hydroxylamine hydrochloride (**1b**),³² *N*-phenyl hydroxylamine (**1c**),³³ *N*-3-chloro-phenyl hydroxylamine (**1d**) and *N*-*t*-butylhydroxylamine hydrochloride (**1e**) (Table 2).

The reaction works well with various isatin ketones **2** and we noticed that the presence of an electron-withdrawing group (Br or NO₂) on C-5 of phenyl ring of isatin does not significantly alters their performance (entries 8–13). Methylation of nitrogen does not reduce the reactivity (entries 4–7). Phenylhydroxylamine **1c** was used in further excess (ratio ketone/phenylhydroxylamine **1** : 3) because of its known degradability at temperature higher than 50 °C.³⁴ Attempts to reduce by-products by lowering power of MW (200 or 400 W) or by reducing the maxim temperature were unnecessary. In all cases the *E*-isomer was the only obtained with the exceptions of compounds **4d** and **4h** for which small quantities of *Z* isomers (ratio *E*/*Z*: 78 : 22 and 75 : 25 for **4d** and **4h**, respectively) were obtained. In general, ketones **3** present lower reactivity with respect to isatin ketones **2**. In all cases, the *Z*-isomer was the only obtained. When an alkyl group is present on the 2-C or 3-C of the carbon backbone of indanone ring (entries 17 and 18), the yield drops presumably due to a minor electrophilicity of carbonyl group. The propyl group introduces additional steric hindrance, and the yield drops to 20% (entry 19).

The presence of Br or F in C-5 of indanone ring (entries 20 and 21) does not produce significant differences of reactivity respect to unsubstituted indanone. On the other hand, the reaction is very sensitive to the size of the cycloalkyl ring (tetralone *versus* indanone). Tetralone derivatives showed very little reactivity and longer reaction times than other substrates (entries 25–27) are required for obtaining just traces of products.

Moreover, resonance effect in C-4 or in C-7 of isatine and indanone ring (entries 14, 22 and 23) causes major reaction times and/or minor yields because the electrophilicity of the acceptor carbonyl group is reduced. Unfortunately, the large size of *t*-butyl group of **1e** dramatically influences both reactions of isatin **2a** and indanone **3a**, not leading to the formation of any product (entries 15 and 24). Attempts to carry out the reactions using the conditions previously applied in entries 7 and 12 of Table 1 confirmed the reaction trend, revealing that these reactions are sensitive to steric effects.

As a follow-up work, some nucleobase-containing spiro-isoxazolidines with an indoline or an indane ring were synthesized by MW-assisted 1,3-dipolar cycloaddition between nitrones as dipole and vinylnucleobases as dipolarophile. It is generally been recognized that the incorporating of different bioactive scaffolds into one molecule is the powerful strategy to

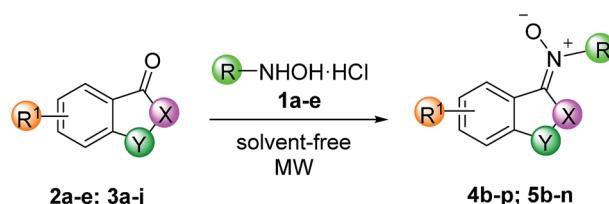
Table 1 Optimization of isatinyl and indanyl nitrones synthesis^a

Entry	Ketone ^b	MW (W)	T (°C)	Time (min)	Product	Yield (%)
1 ^c	2a	200	180	10	4a	33
2 ^c	2a	400	180	10	4a	42
3	2a	400	180	10	4a	53
4	2a	400	180	20	4a	61
5	2a	600	180	10	4a	97
6 ^d	2a	600	180	10	4a	80
7 ^e	2a	—	rt	1440	4a	70
8	3a	600	180	10	5a	37
9	3a	600	180	15	5a	51
10	3a	400	180	10	5a	25
11	3a	400	180	30	5a	82
12 ^d	3a	—	rt	1440	5a	78

^a Reaction conditions: 2.0 eq. of **1a** were used unless otherwise indicated. ^b 1.0 eq. was used. ^c 1.0 eq. of **1a** was used. ^d 1.0 eq. of NaOAc was added. ^e 1 : 1 EtOH/water was used as a solvent and 2.0 eq. of NaOAc were added.



Table 2 Synthesis of nitrones 4 and 5



Entry	Hydroxylamine	R	Ketone	X	Y	R ¹	Time (min)	Product	Yield (%)
1	1b	Bn	2a	C=O	NH	H	12	4b	95
2	1c	Ph ^a	2a	C=O	NH	H	10	4c	92
3	1d	3-Cl-Ph	2a	C=O	NH	H	10	4d	87
4	1a	Me	2b	C=O	NMe	H	10	4e	90
5	1b	Bn	2b	C=O	NMe	H	12	4f	92
6	1c	Ph ^a	2b	C=O	NMe	H	10	4g	90
7	1d	3-Cl-Ph	2b	C=O	NMe	H	10	4h	88
8	1a	Me	2c	C=O	NH	5-Br	10	4i	95
9	1b	Bn	2c	C=O	NH	5-Br	10	4j	93
10	1c	Ph ^a	2c	C=O	NH	5-Br	10	4k	92
11	1a	Me	2d	C=O	NH	5-NO ₂	10	4l	95
12	1b	Bn	2d	C=O	NH	5-NO ₂	13	4m	92
13	1c	Ph ^a	2d	C=O	NH	5-NO ₂	10	4n	92
14	1a	Me	2e	C=O	NH	5,7-Cl	25	4o	80
15	1e	t-Bu	2a	C=O	NH	H	60	4p	—
16	1b	Bn	3a	CH ₂	CH ₂	H	30	5b	85
17	1a	Me	3b	CHMe	CH ₂	H	30	5c	61
18	1a	Me	3c	CH ₂	CHMe	H	35	5d	78
19	1a	Me	3d	CH ⁿ Pr	CH ₂	H	32	5e	20
20	1a	Me	3e	CH ₂	CH ₂	5-Br	30	5f	88
21	1a	Me	3f	CH ₂	CH ₂	5-F	28	5g	83
22	1a	Me	3g	CH ₂	CH ₂	4-Br-7-OH	60	5h	67
23	1b	Bn	3g	CH ₂	CH ₂	4-Br-7-OH	48	5i	78
24	1e	t-Bu	3a	CH ₂	CH ₂	H	60	5j	—
25	1a	Me	3h	CH ₂	CH ₂ CH ₂	H	50	5k	Traces
26	1b	Bn	3h	CH ₂	CH ₂ CH ₂	H	50	5l	Traces
27	1a	Me	3i	CH ₂	CH ₂ CH ₂	7-F	45	5m	Traces
28	1a	Me	3j	CH ₂	CH ₂ CH ₂	6-OMe	55	5n	—

^a Ketone/phenylhydroxylamine ratio 1 : 3.

construct substrates with structural novelty and biological potential.

Considering that, some spiroindoline-isoxazolidines have shown different degrees of anticancer activity mainly based on the isoxazolidine ring fused at C-3 position of oxindole backbone,³⁵⁻³⁷ we hypothesized that the simultaneous presence of an spiro carbon, an oxindole-like ring, an isoxazolidine portion and a nucleobase might generate novel molecular entities with amplified anticancer activity as, for example, inhibitors of MDM2/p53 interaction. Moreover, to our knowledge, in literature spiro-isoxazolidines with nucleobases and indoline or indane ring on their backbone have been never synthesized.

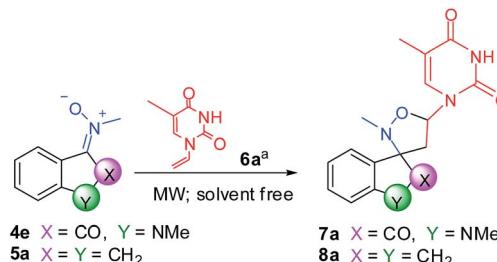
As is well known, the typical insertion of a nucleobase on a sugar or isoxazolidine scaffold consists of the method of Vorbrüggen,³⁸ but for a long time now, we have developed a simple method by solvent-free MW-assisted 1,3-dipolar cycloaddition between nitrones as dipole and vinylnucleobases³⁹ as dipolarophile.⁴⁰

The formation reactions between *N*-1-vinylthymine **6a** and nitrones **4e** and **5a**, respectively, were taken as examples for optimization of reaction conditions. The target conjugates were prepared following the synthetic procedure similar to ketonitrones previously prepared, consisting of the co-grinding of the ketonitrone and vinylnucleobase in a mortar, followed by transfer of the mixture in an appropriate vessel and further mixing of the solids in a vortex without use of solvents; finally the solid mixture was placed in a microwave oven. The results are summarized in Table 3.

We prevalently changed the MW power that, based upon our experience, turns out to be the key parameter in MW-assisted cycloadditions, by keeping the nitrone/vinylnucleobase ratio to 2 : 1, respectively. The optimized conditions for the formation of **7a** are listed in entry 3 (Table 3). On the contrary, the indanone nucleus obliged us to reduce maintained temperature ($T = 125\text{ }^{\circ}\text{C}$) to avoid the formation of a lot quantity of by-products (entries 5–10, Table 3). Despite everything, reaction



Table 3 Optimization of synthesis of nucleobase-containing spiro-isoxazolidines 7a and 8a



Entry	Nitrone ^b	MW (W)	T (°C)	Solvent	Time (min)	Product	Yield (%)
1	4e	650	180	—	30	7a	59
2	4e	750	180	—	20	7a	62
3	4e	850	180	—	10	7a	85
4	4e	—	110	Toluene	4320	7a	25
5	5a	650	180	—	10	8a	22
6	5a	650	80	—	30	8a	34
7	5a	650	125	—	30	8a	38
8	5a	750	80	—	10	8a	41
9	5a	750	100	—	30	8a	61
10	5a	750	125	—	30	8a	77
11	5a	—	110	Toluene	4320	8a	20

^a 1.0 eq. of 6a were used. ^b 2.0 eq. was used.

times were major respect to isatin derivatives and yields were good (entry 10, Table 3). Finally, we compared our results with those obtained from classical conditions of 1,3-dipolar cycloaddition, using toluene and reflux (entries 4 and 11, Table 3). It is possible to highlight that reaction times had to be extended to three days, obtaining very poor yields and many by-products.

Then, the reaction conditions of 7a and 8a were expanded to the formation of some substituted nucleobase-containing spiro-isoxazolidines, using various our nitrones and some vinyl-nucleobases (*N*-1-vinylthymine 6a or *N*-1-vinyluracil 6b). The results are collected in Table 4. All cycloadducts were isolated in good yields and short reaction times (8–30 minutes). The effect of substituents on indoline ring was also investigated, observing slightly yields in presence of bromo or nitro group as substituents because of formation of by-products (entries 5–7, Table 4). All cycloaddition reactions were highly regioselective and goodly diastereoselective, furnishing only one regioisomer (5-substituted) and prevalent *exo*-adduct (Table 4), as confirmed through NOESY experiments. In particular, we observed a specific correlation between the proton on C-6 of thymine or uracil and the proton on C-4 of aromatic ring, only possible in the *exo* approach of cycloaddition.

Theoretical calculations

In order to correlate the reactivity of ketones with hydroxylamines we evaluated the electrophilicity of both reagents by calculating ω reactivity index. The ω index establishes an absolute scale of electrophilicity in the sense that the hierarchy of electrophilicity is built up from the electronic structure of molecules. The electrophilicity hierarchy may be systematically

rationalized on the basis of substituent effects. As a result, electron-withdrawing groups lead to electrophilic activation, and electron-releasing groups lead to electrophilic deactivation. Accordingly, by establishing a scale in which electrophiles and nucleophiles are present, couples of reagents (ketones and hydroxylamines in our case) presenting large differences of electrophilicity (*i.e.* $\Delta\omega > 2.0$) will be the more reactive.

Calculations were carried out at b3lyp-d3bj/def2svp level of theory according to the procedure described elsewhere. We considered the experimentally studied ketones 2a–e and 3a–j but also ketones 2f–k, 3k–3p were calculated in order to predict an extended reactivity. The results are illustrated in Fig. 1.

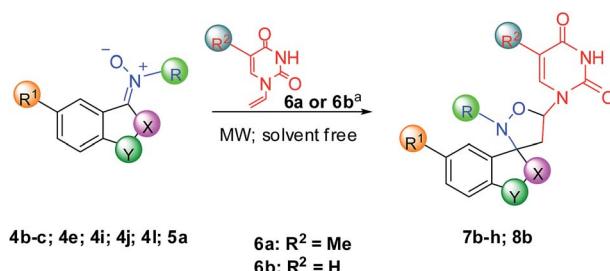
Among the ketones the most electrophilic one is predicted to be 2d, which has been studied experimentally. Among the hydroxylamines the most nucleophilic one resulted 1a, also experimentally studied. Consequently, the reaction between 2d and 1a (Table 2, entry 11) is the most favoured as, indeed, is experimentally demonstrated (high yield and only 10 min of reaction). In general, isatin derivatives are the most reactive with values of $\Delta\omega > 2$ with some exception. The lower reactivity predicted ($\Delta\omega < 2$) for indanyl and tetralonyl derivatives is experimentally confirmed by the longer reaction times required and lower yields obtained. In fact, the absence of product obtained for the reaction between ketone 3j and hydroxylamine 1a correlates with a low difference in electrophilicity ($\Delta\omega = 1.11$).

Experimental

Commercial starting materials were used without further purification. Reactions were monitored by TLC using silica plates 60-F264, commercially available from Merck. ¹H and ¹³C NMR

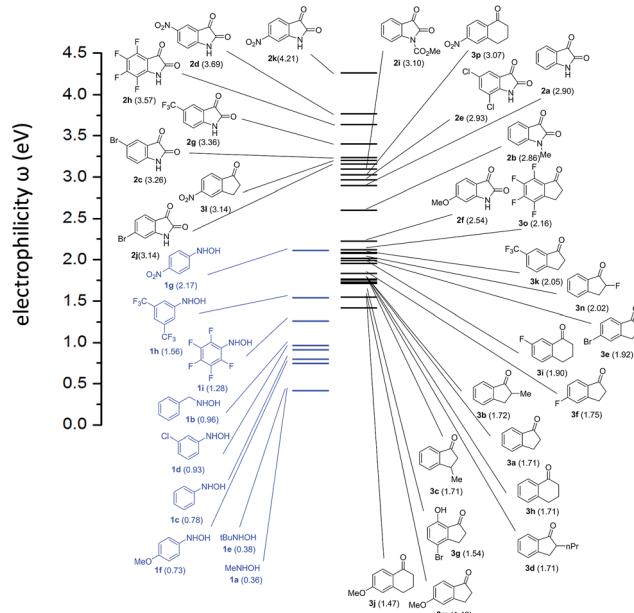


Table 4 MW-assisted synthesis of nucleobase-containing spiro-isoxazolidines



Entry	Nitronate ^{b,c}	R	R ¹	R ²	Time (min)	7,8	Yield (%)	exo/endo ratio
1	4b	Bn	H	H	12	7b	85	83 : 17
2	4c	Ph	H	H	8	7c	84	73 : 27
3	4c	Ph	H	Me	8	7d	85	72 : 28
4	4e^d	Me	H	H	10	7e	84	82 : 18
5	4i	Me	Br	Me	10	7f	77	86 : 14
6	4j	Bn	Br	Me	12	7g	75	88 : 12
7	4l	Bn	NO ₂	Me	12	7h	74	75 : 25
8	5a^e	Me	H	H	30	8b	76	78 : 22

^a 1.0 eq. of **6a** or **6b** were used. ^b 2.0 eq. was used. ^c X = CO; Y = NH unless otherwise indicated. ^d Y = NMe. ^e X = Y = CH₂.

Fig. 1 Electrophilicity (ω in eV) scale for ketones and hydroxylamines.

spectra were recorded at 300, 400 and 500 MHz and 75, 100 and 125 MHz, respectively, in CDCl₃ and DMSO-d₆ using tetramethylsilane (TMS) as internal standard (Bruker ACP 300 MHz, 400 MHz and 500 MHz). Chemical shifts are given in parts per million and coupling constants in Hertz. The stereochemistry were established by NOESY experiments.

LC-MS analysis were carried using an Agilent 6540 UHD Accurate - Mass Q-TOF LC-MS (Agilent, Santa Clara, CA) fitted with a electrospray ionisation source (Dual AJS ESI) operating in positive ion mode. Chromatographic separation was achieved

using a C18 RP analytical column (Poroshell 120, SB-C18, 50 × 2.1 mm, 2.7 μ m) at 30 °C with a elution gradient from 5% to 95% of B over 13 min., a being H₂O (0.1% FA) and B CH₃CN (0.1% FA). Flow rate was 0.4 mL min⁻¹.

MW-assisted reactions were performed in Synthos 3000 instrument from Anton Paar, equipped with a 4 × 24MG5 rotor and an IR probe as external control of the temperature. 0.3–3 mL glass vials sealed with a dedicated PEEK screw-cup together with a reliable PTFE seal were used for all reactions. In synthesis of all derivatives the setting of temperature was always maintained to 180 °C in each experiment, except for **5a–i** where temperature was always maintained to 125 °C.

General procedure for synthesis of nitrones **4a–o** and **5a–i**

The selected ketone (0.5 g) and appropriate hydroxylamine derivative (2 eq. or 3 eq. for *N*-phenylhydroxylamine) were grinded in a mortar, placed in apposite vessel and mixed in a vortex. The mixture was transferred to a microwave oven and was irradiated with the opportune power. After the appropriate time the crude oil was recrystallized with ethyl acetate (**4a–o**) or cyclohexane (**5a–i**).

(E)-N-Methyl-C-isatinyl nitrone 4a. Yellow solid, 97% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 4.28 (s, 3H, CH₃), 6.92 (d, *J* = 7.74 Hz, 1H, Ar), 7.06 (t, *J* = 7.60 Hz, 1H, Ar), 7.38 (t, *J* = 7.60 Hz, 1H, Ar), 8.15 (d, *J* = 7.74 Hz, 1H, Ar), 10.90 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 50.1, 109.1, 117.6, 121.3, 123.2, 130.9, 133.1, 138.9, 160.8. ESI(+)-MS calcd for C₉H₉N₂O₂ [M + H]⁺ 177.0664, found: 177.0655.

(E)-N-Benzyl-C-isatinyl nitrone 4b. Orange solid, 95% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 5.89 (s, 2H, CH₂Bn), 6.91 (d, *J* = 7.78 Hz, 1H, Ar), 7.10 (dt, *J* = 0.91, 7.65 Hz, 1H, Ar), 7.25–7.54 (m, 6H, Ar), 8.12 (d, *J* = 7.65 Hz, 1H, Ar), 11.01 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 64.3, 109.8, 118.1, 122.04, 123.9,



128.5, 129.6, 129.1, 131.8, 133.4, 134.2, 139.6, 161.2. ESI(+)-MS: *m/z* [M + H] calcd for C₁₅H₁₃N₂O₂ 253.0977, found: 253.0977.

(E)-N-Phenyl-C-isatinyl nitrone 4c. Orange red solid, 92% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 6.85 (d, *J* = 7.80 Hz, 1H, Ar), 7.15 (t, *J* = 7.65 Hz, 1H, Ar), 7.40 (t, *J* = 7.80 Hz, 1H, Ar), 7.45–7.65 (m, 5H, Ar), 8.27 (d, *J* = 7.65 Hz, 1H, Ar), 10.78 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 109.7, 118.4, 121.7, 123.9, 124.1, 128.7, 130.1, 132.2, 134.4, 140.9, 146.3, 159.9. ESI(+)-MS: *m/z* [M + H] calcd for C₁₄H₁₁N₂O₂ 239.0821, found: 239.0817.

(E)-N-3-Cl-phenyl-C-isatinyl nitrone 4d (the most abundant). Orange solid, 87% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 6.35 (d, *J* = 7.71, 1H, Ar), 6.79 (t, *J* = 7.71, 1H, Ar), 6.85–7.05 (m, 2H, Ar), 7.07–7.18 (m, 1H, Ar), 7.27–7.44 (m, 2H, Ar), 7.45–7.56 (m, 1H, Ar), 11.04 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 111.6, 115.6, 116.1, 117.1, 121.9, 124.6, 125.4, 131.4, 134.0, 134.6, 146.0, 150.8, 155.6, 163.3. ESI(+)-MS: *m/z* [M + H] calcd for C₁₄H₁₀ClN₂O₂ 273.0431, found: 273.0422; [M + H] + 2 calcd for C₁₄H₁₀ClN₂O₂ 275.0401, found: 275.0382.

(E)-N-Methyl-C-(1-methyl)-isatinyl nitrone 4e. Yellow solid, 90% yield. ¹H NMR (300 MHz, CDCl₃): δ 3.27 (s, 3H, CH₃), 4.38 (s, 3H, CH₃), 6.82 (d, *J* = 7.83 Hz, 1H, Ar), 7.09 (t, *J* = 7.65 Hz, 1H, Ar), 7.38 (t, *J* = 7.83 Hz, 1H, Ar), 8.30 (d, *J* = 7.65 Hz, 1H, Ar), ¹³C NMR (75 MHz, CDCl₃): δ 26.0, 51.2, 107.6, 118.3, 123.0, 123.0, 124.8, 131.3, 141.2, 160.9. ESI(+)-MS: *m/z* [M + H] calcd for C₁₀H₁₁N₂O₂ 191.0821, found: 191.0815.

(E)-N-Benzyl-C-(1-methyl)-isatinyl nitrone 4f. Yellow solid, 92% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 3.25 (s, 3H, CH₃), 5.92 (s, 2H, CH₂), 7.08 (t, *J* = 8.26 Hz, 2H, Ar), 7.37–7.50 (m, 6H, Ar), 8.15 (d, *J* = 7.34 Hz, 1H, Ar), ¹³C NMR (75 MHz DMSO-d₆): δ 26.6, 65.2, 109.2, 117.8, 123.1, 124.0, 129.0, 129.0, 129.59, 132.2, 134.6, 141.5, 160.4. ESI(+)-MS: *m/z* [M + H] calcd for C₁₆H₁₅N₂O₂ 267.1134, found: 267.1126.

(E)-N-Phenyl-C-(1-methyl)-isatinyl nitrone 4g. Orange solid, 90% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 3.11 (s, 3H, CH₃), 7.08–7.16 (m, 2H, Ar), 7.46–7.58 (m, 6H, Ar), 8.30 (d, *J* = 7.48 Hz, 1H, Ar), ¹³C NMR (75 MHz, DMSO-d₆): δ 26.5, 110.7, 115.4, 116.5, 117.5, 122.8, 125.1, 125.5, 131.9, 134.4, 135.1, 148.5, 152.2, 155.3, 162.4. ESI(+)-MS: *m/z* [M + H] calcd for C₁₅H₁₃N₂O₂ 253.0977, found: 253.0975.

(E)-N-3-Cl-phenyl-C-(1-methyl)-isatinyl nitrone 4h (the most abundant). Yellow solid, 88% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 3.21 (s, 3H, CH₃), 6.37–6.40 (m, 1H, Ar), 6.85–7.12 (m, 4H, Ar), 7.32–7.65 (m, 3H, Ar), ¹³C NMR (75 MHz, DMSO-d₆): δ 26.6, 110.7, 115.4, 116.5, 117.5, 122.8, 125.1, 125.5, 131.9, 134.4, 135.1, 148.5, 152.2, 155.3, 162.4. ESI(+)-MS: *m/z* [M + H] calcd for C₁₅H₁₂ClN₂O₂ 287.0587, found: 287.0577; [M + H] + 2 calcd for C₁₅H₁₂ClN₂O₂ 289.0558, found: 289.0557.

(E)-N-Methyl-C-(5-Br)-isatinyl nitrone 4i. Orange solid, 95% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 4.26 (s, 3H, CH₃), 6.83 (d, *J* = 8.24 Hz, 1H, Ar), 7.50 (dd, *J* = 1.92 Hz, 8.24, 1H, Ar), 8.18 (d, *J* = 1.92 Hz, 1H, Ar), 11.02 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 51.4, 112.0, 113.7, 120.4, 126.0, 133.5, 134.0, 139.0, 161.4. ESI(+)-MS: *m/z* [M + H] calcd for C₉H₈BrN₂O₂ 254.9769, found: 254.9762; [M + H] + 2 calcd for C₉H₈BrN₂O₂ 256.9749, found: 256.9746.

(E)-N-Benzyl-C-(5-Br)-isatinyl nitrone 4j. Orange solid, 93% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 5.89 (s, 2H, CH₂), 6.87 (d,

J = 7.95 Hz, 1H, Ar), 7.26–7.63 (m, 6H, Ar), 8.21 (s, 1H, Ar), 11.16 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 65.2, 112.2, 113.9, 120.5, 126.2, 129.0, 129.5, 133.3, 134.4, 134.4, 139.2, 161.3. ESI(+)-MS: *m/z* [M + H] calcd for C₁₅H₁₂BrN₂O₂ 331.0082, found: 333.0071; [M + H] + 2 calcd for C₁₅H₁₂BrN₂O₂ 333.0062, found: 333.0053.

(E)-N-Phenyl-C-(5-Br)-isatinyl nitrone 4k. Orange solid, 92% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 6.86 (dd, *J* = 2.93 Hz, 8.25 Hz, 1H, Ar), 7.40–7.77 (m, 6H, Ar), 8.37 (d, *J* = 1.83 Hz, 1H, Ar), 10.92 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 116.9, 118.4, 125.6, 129.1, 131.2, 134.0, 135.7, 135.8, 139.6, 145.3, 151.4, 164.8. ESI(+)-MS: *m/z* [M + H] calcd for C₁₄H₁₀BrN₂O₂ 316.9932, found: 316.9919; [M + H] + 2 calcd for C₁₄H₁₀BrN₂O₂ 318.9905, found: 318.9899.

(E)-N-Methyl-C-(5-NO₂)-isatinyl nitrone 4l. Yellow solid, 95% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 4.28 (s, 3H, CH₃), 7.02 (d, *J* = 8.73 Hz, 1H, Ar), 8.23 (d, *J* = 8.73 Hz, 1H, Ar), 8.75 (s, 1H, Ar), 11.53 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 51.8, 110.2, 118.7, 118.7, 128.0, 133.3, 142.4, 145.2, 161.9. ESI(+)-MS: *m/z* [M + H] calcd for C₉H₈N₃O₄ 222.0515, found: 222.0503.

(E)-N-Benzyl-C-(5-NO₂)-isatinyl nitrone 4m. Yellow solid, 92% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 5.91 (s, 2H, CH₂), 7.07 (d, *J* = 8.73 Hz, 1H, Ar), 7.31–7.58 (m, 5H, Ar), 8.28 (dd, *J* = 2.43 Hz, 8.73 Hz, 1H, Ar), 8.84 (d, *J* = 2.43 Hz, 1H, Ar), 11.70 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 65.6, 110.4, 118.8, 119.0, 128.3, 129.1, 129.6, 133.2, 134.1, 142.6, 145.4, 161.9. ESI(+)-MS: *m/z* [M + H] calcd for C₁₅H₁₂N₃O₄ 298.0828, found: 298.0816.

(E)-N-Phenyl-C-(5-NO₂)-isatinyl nitrone 4n. Dark yellow solid, 92% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 7.06 (d, *J* = 8.79 Hz, 1H, Ar), 7.46–7.69 (m, 5H, Ar), 8.33 (dd, *J* = 2.37 Hz, 8.79 Hz, 1H, Ar), 9.00 (d, *J* = 1.83 Hz, 1H, Ar), 11.47 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 110.3, 119.2, 124.4, 128.8, 129.8, 131.2, 134.2, 142.4, 146.5, 146.7, 160.6. ESI(+)-MS: *m/z* [M + H] calcd for C₁₄H₁₀N₃O₄ 284.0671, found: 284.0662.

(E)-N-Methyl-C-(5,7-Cl)-isatinyl nitrone 4o. Orange solid, 80% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 4.25 (s, 3H, CH₃), 7.47 (d, *J* = 2.01 Hz, 1H, Ar), 7.91 (d, *J* = 2.01 Hz, 1H, Ar), 11.37 (s, 1H, NH), ¹³C NMR (75 MHz, DMSO-d₆): δ 51.8, 115.0, 121.0, 121.8, 126.6, 130.2, 133.5, 136.2, 161.4. ESI(+)-MS: *m/z* [M + H] calcd for C₉H₇Cl₂N₂O₂ 244.9885, found: 244.9878; [M + H] + 2 calcd for C₉H₇Cl₂N₂O₂ 246.9855, found: 246.9849; [M + H] + 4 calcd for C₉H₇Cl₂N₂O₂ 248.9826, found: 248.9820.

(Z)-N-Methyl-C-indanyl nitrone 5a. White solid, 82% yield. ¹H NMR (300 MHz, CDCl₃): δ 2.90–3.05 (m, 2H, CH₂), 3.11–3.22 (m, 2H, CH₂), 3.81 (s, 3H, CH₃), 7.29–7.44 (m, 3H, Ar), 8.84 (d, *J* = 7.81 Hz, 1H, Ar), ¹³C NMR (75 MHz, CDCl₃): δ 28.7, 29.4, 49.6, 124.5, 127.0, 131.0, 134.4, 147.8, 149.7. ESI(+)-MS: *m/z* [M + H] calcd for C₁₀H₁₂NO 162.0919, found: 162.0912.

(Z)-N-Benzyl-C-indanyl nitrone 5b. White solid, 85% yield. ¹H NMR (300 MHz, CDCl₃): δ 2.91–3.09 (m, 2H, CH₂), 3.10–3.23 (m, 2H, CH₂), 5.11 (s, 2H, CH₂Bn), 7.21–7.45 (m, 7H, Ar), 7.51 (d, *J* = 7.16 Hz, 1H, Ar), 8.92 (d, *J* = 7.63 Hz, 1H, Ar), ¹³C NMR (75 MHz, CDCl₃): δ 29.0, 29.2, 66.6, 124.5, 127.1, 127.2, 128.2, 128.3, 128.9, 131.1, 133.4, 134.8, 147.7, 149.1. ESI(+)-MS: *m/z* [M + H] calcd for C₁₆H₁₆NO 238.1232, found: 238.1230.





(Z)-N-Methyl-C-(2-Me)-indanyl nitrone 5c. White solid, 61% yield. ^1H NMR (300 MHz, CDCl_3): δ 1.28 (d, $J = 6.99$ Hz, 3H, CH_3), 2.70 (d, $J = 16.5$ Hz, 1H, H_{CH_2}), 3.20–3.50 (m, 2H, $\text{CH} + \text{H}_{\text{CH}_2}$), 3.85 (s, 3H, CH_3), 7.22–7.48 (m, 3H, Ar), 8.83 (d, $J = 7.53$ Hz, 1H, Ar), ^{13}C NMR (75 MHz, CDCl_3): δ 19.7, 36.4, 38.4, 48.9, 124.7, 127.1, 127.5, 131.1, 133.5, 145.9, 153.8. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{11}\text{H}_{14}\text{NO}$ 176.1075, found: 176.1068.

(Z)-N-Methyl-C-(3-Me)-indanyl nitrone 5d. White solid, 78% yield. ^1H NMR (300 MHz, CDCl_3): δ 1.38 (d, 3H, $J = 7.02$ Hz, CH_3), 2.53 (dd, 1H, $J = 2.79$ Hz, 17.76 Hz, H_{CH_2}), 3.24 (dd, 1H, $J = 7.29$ Hz, 17.76 Hz, H_{CH_2}), 3.40–3.58 (m, 1H, CH), 3.78 (s, 3H, CH_3), 7.28–7.51 (m, 3H, Ar), 8.83 (d, $J = 7.68$ Hz, 1H, Ar), ^{13}C NMR (75 MHz, CDCl_3): δ 21.7, 35.9, 38.8, 49.8, 123.5, 126.9, 127.3, 131.2, 133.8, 152.6. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{11}\text{H}_{14}\text{NO}$ 176.1075, found: 176.1071.

(Z)-N-Methyl-C-(2-Pr)-indanyl nitrone 5e. White solid, 20% yield. ^1H NMR (300 MHz, CDCl_3): δ 0.90 (t, 3H, $J = 6.47$ Hz, CH_3), 1.25–155 (m, 4H, CH_2), 2.90–3.42 (m, 3H, $\text{CH} + \text{CH}_2$), 3.83 (s, 3H, CH_3), 7.18–7.49 (m, 3H, Ar), 8.79 (d, $J = 7.51$ Hz, 1H, Ar), ^{13}C NMR (75 MHz, CDCl_3): δ 13.8, 20.0, 35.7, 35.9, 41.6, 48.8, 124.7, 127.0, 127.5, 131.3, 134.0, 134.5, 146.7. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{13}\text{H}_{18}\text{NO}$ 204.1388, found: 204.1384.

(Z)-N-Methyl-C-(5-Br)-indanyl nitrone 5f. White solid, 88% yield. ^1H NMR (300 MHz, CDCl_3): δ 3.0 (dd, $J = 5.55$ Hz, 12.03 Hz, 2H, CH_2), 3.14 (dd, $J = 5.55$ Hz, 12.51 Hz, 2H, CH_2), 3.81 (s, 3H, CH_3), 7.29–7.44 (m, 3H, Ar), 8.84 (d, $J = 7.81$ Hz, 1H, Ar), ^{13}C NMR (75 MHz, CDCl_3): δ 28.6, 29.5, 49.9, 125.1, 127.8, 128.0, 130.4, 133.6, 148.1, 149.4. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{10}\text{H}_{11}\text{BrNO}$ 240.0024, found: 240.0020; [M + H] + 2 calcd for $\text{C}_{10}\text{H}_{11}\text{BrNO}$ 242.0004, found: 242.0000.

(Z)-N-Methyl-C-(5-F)-indanyl nitrone 5g. White solid, 83% yield. ^1H NMR (300 MHz, CDCl_3): δ 3.01 (dd, $J = 5.43$ Hz, 11.73 Hz, 2H, CH_2), 3.14 (dd, $J = 5.55$ Hz, 11.97 Hz, 2H, CH_2), 6.80–7.12 (m, 2H, Ar), 8.86 (dd, $J = 5.79$ Hz, 8.64 Hz, 1H, Ar), ^{13}C NMR (75 MHz, CDCl_3): δ 28.8 (d, $J_{\text{CF}} = 2.28$), 29.9, 49.6, 111.9 (d, $J^2_{\text{CF}} = 23.02$), 114.3 (d, $J^2_{\text{CF}} = 22.86$), 128.8 (d, $J^3_{\text{CF}} = 9.03$), 130.9 (d, $J^4_{\text{CF}} = 2.02$), 148.1, 150.5 (d, $J^3_{\text{CF}} = 8.95$), 164.43 (d, $J^1_{\text{CF}} = 250.96$). ESI(+)MS: m/z [M + H] calcd for $\text{C}_{10}\text{H}_{11}\text{FNO}$ 180.0825, found: 180.0818.

(Z)-N-Methyl-C-(4-Br-7-OH)-indanyl nitrone 5h. Yellow solid, 67% yield. ^1H NMR (300 MHz, CDCl_3): δ 2.86–2.95 (m, 2H, CH_2), 2.96–3.30 (m, 2H, CH_2), 3.70 (s, 3H, CH_3), 6.60 (d, 1H, $J = 8.79$ Hz, Ar), 7.37 (d, $J = 8.79$ Hz, 1H, Ar), 14.96 (s, 1H, OH), ^{13}C NMR (75 MHz, CDCl_3): δ 30.2, 47.8, 107.2, 118.5, 121.2, 138.0, 149.6, 155.4, 157.9. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{10}\text{H}_{11}\text{BrNO}_2$ 255.9973, found: 255.9964; [M + H] + 2 calcd for $\text{C}_{10}\text{H}_{11}\text{BrNO}_2$ 255.9953, found: 255.9945.

(Z)-N-Benzyl-C-(4-Br-7-OH)-indanyl nitrone 5i. Pale yellow solid, 78% yield. ^1H NMR (300 MHz, CDCl_3): δ 2.97 (s, 4H, CH_2), 5.04 (s, 2H, CH_2), 6.62 (d, 1H, $J = 8.79$ Hz, Ar), 7.35–7.50 (m, 6H, Ar), 15.02 (s, 1H, OH), ^{13}C NMR (75 MHz, CDCl_3): δ 29.8, 30.2, 64.6, 107.1, 118.6, 121.3, 127.9, 128.8, 129.1, 132.4, 138.0, 149.6, 155.5, 158.1. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{16}\text{H}_{15}\text{BrNO}_2$ 332.0286, found: 332.0276; [M + H] + 2 calcd for $\text{C}_{16}\text{H}_{15}\text{BrNO}_2$ 334.0266, found 334.0259.

General procedure for synthesis of nucleobase-containing spiro-isoxazolidines 7a–h and 8a–b

The opportune nitrone (2 eq.) and *N*-1-vinylthymine **6a** or *N*-1-vinyluracil **6b** (0.05 g) were grinded in a mortar, placed in apposite vessel and mixed in a vortex. The mixture was transferred to a microwave oven and was irradiated with the opportune power and temperature (when required). After the appropriate time the crude oil was purified by flash chromatography with hexane : AcOEt 7.75 : 2.25 v/v for **7c–e** or CHCl_3 / MeOH 9.75 : 0.25 v/v for all the other crudes.

exo-5'-Thyminyl-2'-methyl-spiro-[indoline-3,3'-isoxazolidine]-1-methyl-2-one 7a. Red solid, 85% yield. ^1H NMR (500 MHz, CDCl_3): δ 2.02 (d, $J = 1.21$ Hz, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.64 (dd, $J = 4.31$ Hz, 14.03 Hz, 1H, H_{CH_2}), 3.22 (s, 3H, CH_3), 3.28 (dd, $J = 7.48$ Hz, 14.03 Hz, 1H, H_{CH_2}), 6.44 (dd, $J = 4.31$ Hz, 7.48 Hz, 1H, CH), 6.86 (d, $J = 7.85$ Hz, 1H, Ar), 7.09–7.14 (m, 1H, Ar), 7.24–7.29 (m, 1H, Ar), 7.36–7.41 (m, 1H, Ar), 7.77 (d, $J = 1.21$ Hz, 1H, 6- CH_{Thy}), 8.65 (s_b, 1H, NH_{Thy}). ^{13}C NMR (125 MHz, CDCl_3): δ 12.8, 26.0, 38.0, 47.6, 72.3, 83.4, 108.6, 110.7, 123.3, 123.3, 124.1, 130.5, 135.3, 144.2, 150.2, 163.8, 173.9. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{17}\text{H}_{19}\text{N}_4\text{O}_4$ 343.1406, found: 343.1403.

exo-5'-Uracil-2'-benzyl-spiro-[indoline-3,3'-isoxazolidine]-2-one 7b. Yellow solid, 85% yield. ^1H NMR (300 MHz, DMSO-d_6): δ 2.76–289 (m, 1H, H_{CH_2}), 3.41–3.58 (m, 1H, H_{CH_2}), 5.05–5.14 (m, 2H, $\text{CH}_{2\text{Bn}}$), 5.59 (dd, $J = 2.20$ Hz, 7.95 Hz, 1H, CH), 6.68 (d, $J = 7.68$ Hz, 1H, 5- CH_{Ura}), 6.75–7.92 (m, 9H, Ar), 7.97 (d, $J = 7.68$, 1H, 6- CH_{Ura}), 10.23 (s_b, 1H, NH), 11.05 (s_b, 1H, NH_{Ura}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 61.4, 72.6, 101.2, 109.9, 122.0, 125.3, 127.1, 127.3, 128.1, 128.4, 128.9, 129.5, 130.2, 142.8, 143.4, 151.2, 163.1, 168.5, 180.2. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{21}\text{H}_{19}\text{NaN}_4\text{O}_4$ 413.1226, found: 413.1219.

exo-5'-Uracil-2'-phenyl-spiro-[indoline-3,3'-isoxazolidine]-2-one 7c. Orange solid, 84% yield. ^1H NMR (300 MHz, DMSO-d_6): δ 2.98–3.11 (m, 1H, H_{CH_2}), 3.25–3.33 (m, 1H, H_{CH_2}), 5.79 (d, $J = 8.07$ Hz, 1H, 5- CH_{Ura}), 6.24 (dd, $J = 5.31$ Hz, 7.14 Hz, 1H, CH), 6.65–7.66 (m, 8H, Ar), 7.98 (d, $J = 8.28$ Hz, 1H, Ar), 8.55 (d, $J = 8.07$ Hz, 1H, 6- CH_{Ura}), 10.43 (s_b, 1H, NH), 10.89 (s_b, 1H, NH_{Ura}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.8, 72.9, 80.9, 102.6, 110.5, 118.1, 124.4, 126.1, 128.8, 128.9, 128.2, 130.9, 132.7, 141.8, 145.6, 151.4, 163.4, 175.6. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{20}\text{H}_{17}\text{N}_4\text{O}_4$ 377.1250, found: 377.1237.

exo-5'-Thyminyl-2'-phenyl-spiro-[indoline-3,3'-isoxazolidine]-2-one 7d. Orange solid, 85% yield. ^1H NMR (300 MHz, DMSO-d_6): δ 1.89 (s, 3H, CH_3), 2.99–3.14 (m, 1H, H_{CH_2}), 3.16–3.32 (m, 1H, H_{CH_2}), 6.67–7.58 (m, 10H, Ar + CH), 8.53 (s, 1H, 6- CH_{Thy}), 10.46 (s_b, 1H, NH), 11.48 (s_b, 1H, NH_{Thy}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.8, 45.0, 73.5, 81.8, 100.3, 110.8, 117.3, 118.0, 122.8, 124.6, 128.8, 128.9, 130.9, 135.7, 142.8, 146.4, 151.1, 164.0, 175.8. ESI(+)MS: m/z [M + H] calcd for $\text{C}_{21}\text{H}_{19}\text{N}_4\text{O}_4$ 391.1406, found: 391.1402.

exo-5'-Uracil-2'-methyl-spiro-[indoline-3,3'-isoxazolidine]-1-methyl-2-one 7e. Orange solid, 84% yield. ^1H NMR (300 MHz, DMSO-d_6): δ 2.42 (s, 3H, CH_3), 2.82 (dd, $J = 4.86$ Hz, 13.98 Hz, 1H, H_{CH_2}), 3.05–3.22 (m, 4H, $\text{CH}_3 + \text{H}_{\text{CH}_2}$), 6.34 (dd, $J = 4.86$ Hz, 7.47 Hz, 1H, CH), 7.00–7.21 (m, 2H, Ar), 7.38–7.55 (m, 2H, Ar), 7.98 (d, $J = 8.16$ Hz, 1H, 5- CH_{Ura}), 8.03 (d, $J = 8.16$ Hz, 1H, 6- CH_{Ura}),

11.46 (s_b, 1H, NH_{Ura}). ¹³C NMR (75 MHz, DMSO-d₆): δ 26.3, 38.1, 46.3, 72.5, 82.6, 108.9, 109.5, 123.2, 124.9, 129.9, 130.7, 140.1, 144.7, 151.0, 163.7, 173.9. ESI(+)-MS: *m/z* [M + H] calcd for C₁₆H₁₆N₄O₄ 329.1250, found: 329.1242.

***exo*-5'-Thyminyl-2'-methyl-spiro-[5-bromo-indoline-3,3'-isoxazolidine]-2-one 7f.** Yellow solid, 77% yield. ¹H NMR (500 MHz, DMSO-d₆): δ 1.92 (s, 3H, CH₃), 2.97 (dd, *J* = 6.04 Hz, 13.73 Hz, 1H, H_{CH₂}), 3.46 (dd, *J* = 7.14 Hz, 13.73 Hz, 1H, H_{CH₂}), 3.54 (s, 3H, NCH₃), 6.40 (dd, *J* = 6.04 Hz, 7.14 Hz, 1H, CH), 6.85 (d, *J* = 8.23 Hz, 1H, Ar), 7.18–7.56 (m, 2H, Ar), 7.72–7.81 (m, 2H, Ar + 6-CH_{Thy}), 10.78 (s_b, 1H, NH), 11.38 (s_b, 1H, NH_{Thy}). ¹³C NMR (125 MHz, DMSO-d₆): δ 16.7, 33.3, 42.4, 74.2, 86.3, 114.1, 116.3, 118.1, 129.9, 130.9, 137.2, 140.0, 146.5, 154.9, 168.2, 179.3. ESI(+)-MS: *m/z* [M + H] calcd for C₁₆H₁₆BrN₄O₄ 407.0355, found: 407.0337; [M + H] + 2 calcd for C₁₆H₁₆BrN₄O₄ 409.0334, found: 409.0322.

***exo*-5'-Thyminyl-2'-benzyl-spiro-[5-bromo-indoline-3,3'-isoxazolidine]-2-one 7g.** Yellow solid, 75% yield. ¹H NMR (500 MHz, DMSO-d₆): δ 1.82 (s, 3H, CH₃), 2.8–3.2 (m, 2H, H_{CH₂}), 4.35 (s, 2H, CH_{2Bn}), 6.84 (dd, *J* = 6.24 Hz, 8.85 Hz, 1H, CH), 6.70–8.71 (m, 8H, Ar), 8.98 (d, *J* = 5.42 Hz, 1H, 6-CH_{Thy}), 11.10 (s_b, 1H, NH), 11.36 (s_b, 1H, NH_{Thy}). ¹³C NMR (125 MHz, DMSO-d₆): δ 16.3, 33.3, 39.5, 67.0, 76.2, 113.6, 116.6, 117.7, 126.4, 132.1, 132.5, 132.9, 133.5, 137.8, 142.5, 144.4, 146.6, 149.4, 155.6, 167.6. ESI(+)-MS: *m/z* [M + H] calcd for C₂₂H₂₀BrN₄O₄ 483.0668, found: 483.0666; [M + H] + 2 calcd for C₂₂H₂₀BrN₄O₄ 485.0647, found: 485.0637.

***exo*-5'-Thyminyl-2'-benzyl-spiro-[5-nitro-indoline-3,3'-isoxazolidine]-2-one 7h.** Orange solid, 74% yield. ¹H NMR (300 MHz, DMSO-d₆): δ 1.79 (s, 3H, CH₃), 3.54 (dd, *J* = 7.03 Hz, 14.22 Hz, 1H, H_{CH₂}), 3.93–4.19 (m, 3H, H_{CH₂} + CH_{2Bn}), 5.48 (dd, *J* = 7.03 Hz, 9.72 Hz, 1H, CH), 6.92 (d, *J* = 8.52 Hz, 1H, Ar), 7.20–7.78 (m, 6H, Ar), 8.15 (dd, *J* = 2.35 Hz, 8.70, 1H, Ar), 8.23 (d, *J* = 2.19 Hz, 1H, 6-CH_{Thy}), 11.03 (s_b, 1H, NH), 11.12 (s_b, 1H, NH_{Thy}). ¹³C NMR (75 MHz, DMSO-d₆): δ 12.6, 22.5, 29.4, 58.7, 71.2, 108.8, 109.9, 120.5, 126.4, 127.1, 128.7, 132.0, 132.8, 137.8, 142.8, 145.0, 148.9, 151.5, 163.7, 180.3. ESI(+)-MS: *m/z* [M + H] calcd for C₂₂H₂₀N₅O₆ 450.1414, found: 450.1403.

***exo*-5'-Thyminyl-2'-methyl-spiro-[indane-3,3'-isoaxazolidine] 8a.** White solid, 77% yield. ¹H NMR (400 MHz, CDCl₃): δ 1.76–1.90 (m, 2H, CH₂), 1.94 (d, *J* = 1.08 Hz, 1H, CH₃), 2.38 (s, 3H, CH₃), 2.51–2.67 (m, 2H, CH₂), 2.79–3.05 (m, 2H, CH₂), 6.20 (dd, *J* = 5.68 Hz, 7.36 Hz, 1H, CH), 7.12–7.35 (m, 4H, Ar), 7.79 (s, 1H, 6-CH_{Thy}), 9.39 (s_b, 1H, NH_{Thy}). ¹³C NMR (100 MHz, CDCl₃): δ 12.8, 29.2, 30.2, 36.1, 51.6, 77.9, 82.2, 111.1, 123.6, 125.2, 126.9, 129.0, 135.9, 139.8, 144.3, 150.7, 164.2. ESI(+)-MS: *m/z* [M + H] calcd for C₁₇H₂₀N₃O₃ 314.1505, found: 314.1497.

***exo*-5'-Uracil-2'-methyl-spiro-[indane-3,3'-isoaxazolidine] 8b.** White solid, 76% yield. ¹H NMR (400 MHz, CDCl₃): δ 1.78–1.95 (m, 2H, CH₂), 2.39 (s, 3H, CH₃), 2.52–2.72 (m, 2H, CH₂), 2.79–3.10 (m, 2H, CH₂), 5.77 (d, *J* = 8.02 Hz, 1H, 5-CH_{Ura}), 6.19 (dd, *J* = 5.38 Hz, 7.24 Hz, 1H, CH), 7.10–7.40 (m, 4H, Ar), 8.05 (d, *J* = 8.02 Hz, 1H, 6-CH_{Ura}), 9.53 (s_b, 1H, NH_{Ura}). ¹³C NMR (100 MHz, CDCl₃): δ 29.4, 30.1, 35.9, 78.0, 82.6, 102.6, 123.6, 125.1, 127.0, 129.1, 139.5, 140.3, 144.3, 150.6, 163.7. ESI(+)-MS: *m/z* [M + H] calcd for C₁₆H₁₈N₃O₃ 300.1348, found: 300.1337.

Conclusions

In summary, we have initially developed an environmentally benign and fast method by microwave irradiation and solvent-free conditions for the synthesis of significant number of substituted isatinyl and indanyl nitrones as important precursors of spiro-isoxazolidines. Then, we applied a MW-assisted 1,3-dipolar cycloaddition to some previously synthesized ketonitrones as dipole and vinylthymine or vinyluracil as dipolarophile, obtaining nucleobase-containing spiro-isoxazolidines with indoline or indane ring in good yields, excellent regiosomery and high diastereoisomery. The importance of this application is surely the direct insertion of a nucleobase on spiroisoxazolidines with indoline or indane scaffold that in our opinion represents a great chemical novelty. Finally, we have also enriched this work, studying by theoretical calculations the effect of various substituents about the reactivity of the carbonyl groups with different hydroxylamines in synthesis of nitrones.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- 1 C. V. Galliford and K. A. Scheidt, *Angew. Chem., Int. Ed.*, 2007, **46**, 8748.
- 2 D. Cheng, Y. Ishihara, C. F. Barbas III and B. Tan, *ACS Catal.*, 2014, **4**, 743.
- 3 B. Tan, X. Zeng, W. W. Y. Leong, Z. Shi, C. F. Barbas III and G. Zhong, *Chem.– Eur. J.*, 2012, **18**, 63.
- 4 M. C. Pirrung, S. V. Pansare, K. D. Sarma, K. A. Keith and E. R. Kern, *J. Med. Chem.*, 2005, **48**, 3045.
- 5 D. J. Bauer and P. W. Sadler, *Nature*, 1961, **190**, 1167.
- 6 J. M. Z. Gladich, J. H. Hunt, D. Jack, R. F. Haff, J. J. Boyle, R. C. Stewart and R. J. Ferlauto, *Nature*, 1969, **221**, 286.
- 7 D. Sriram, T. R. Bal and P. Yogeeshwari, *J. Pharm. Pharm. Sci.*, 2005, **8**, 565.
- 8 K. Ding, Y. Lu, Z. Nikolovska-Coleska, G. Wang, S. Qiu, S. Shangary, W. Gao, D. Qin, J. Stuckey, K. Krajewski, P. P. Roller and S. Wang, *J. Med. Chem.*, 2006, **49**, 3432.
- 9 S. Wang, Y. Zhao, D. Bernard, A. Aguilar and S. Kumar, *Top. Med. Chem.*, 2012, **8**, 57.
- 10 F. Shi, Z.-L. Tao, S.-W. Luo, S.-J. Tu and L.-Z. Gong, *Chem.– Eur. J.*, 2012, **18**, 6885.



11 C.-S. Wang, R.-Y. Zhu, J. Zheng, F. Shi and S.-J. Tu, *J. Org. Chem.*, 2015, **80**, 512.

12 W. Dai, X.-L. Jiang, Q. Wu, F. Shi and S.-J. Tu, *J. Org. Chem.*, 2015, **80**, 5737.

13 C. Chavarria, D. I. Perez, C. Perez, J. Morales-Garcia, S. Alonso-Gil, A. Perez Castillo, C. Gil, J. Souza and W. Porcal, *Eur. J. Med. Chem.*, 2012, **58**, 44.

14 V. Gautheron-Chapoulaud, S. U. Pandya, P. Cividino, G. Masson, S. Py and Y. Vallée, *Synlett*, 2001, **8**, 1281.

15 G. Masson, S. Py and Y. Vallée, *Angew. Chem., Int. Ed.*, 2002, **41**, 1772.

16 H. I. Petkes, E. Gal, L. Gaina, M. Sabou, C. Majdik and L. Silaghi-Dumitrescu, *C. R. Chim.*, 2014, **17**, 1050.

17 G. Soldaini, F. Cardona and A. Goti, *Org. Lett.*, 2007, **9**, 473.

18 S. Franco, F. L. Merchant, P. Merino and T. Tejero, *Synth. Commun.*, 1995, **25**, 2275.

19 Y. Tomioka, C. Nagahiro, Y. Nomura and H. Maruoka, *J. Heterocycl. Chem.*, 2003, **40**, 121.

20 R. Y. Suman, P. Kadigachalam, V. R. Doddi and Y. D. Vankar, *Tetrahedron Lett.*, 2009, **50**, 5827.

21 H. G. Aurich and W. Weiss, *Tetrahedron*, 1976, **32**, 159.

22 K. Torsell and O. Zeuthen, *Acta Chem. Scand., Ser. A*, 1978, **32**, 118.

23 H. Valizadeh, *Heteroat. Chem.*, 2010, **21**, 78.

24 H.-B. Yang and S. Min, *Org. Biomol. Chem.*, 2012, **10**, 8236.

25 Y.-H. Zhang, M.-Y. Wu and W.-C. Huang, *RSC Adv.*, 2015, **5**, 105825.

26 I. S. Young and M. A. Kerr, *Angew. Chem., Int. Ed.*, 2003, **26**, 3023.

27 S.-Y. Wu, X.-P. Ma, C. Liang and D.-L. Mo, *J. Org. Chem.*, 2017, 3232.

28 A. Loupy, in *Microwave in Organic Synthesis*, Wiley-VCH, Weinheim, 2002.

29 J. P. Tieney and P. Lidstrom, in *Microwave Assisted Organic Chemistry*, Blackwell Publishing, Oxford, 2005.

30 L. Maiuolo, A. De Nino, P. Merino, B. Russo, G. Stabile, M. Nardi, N. D'Agostino and T. Bernardi, *Arabian J. Chem.*, 2016, **9**, 25.

31 F. M. Murphy and M. J. Meegan, *J. Chem. Res., Miniprint*, 1996, 301.

32 T. B. Nguyen, A. Martel, R. Dhal and G. Dujardin, *Synthesis*, 2009, **18**, 3174.

33 B. S. Furniss; A. J. Hannaford; P. W. G. Smith and A. R. Tatchell, in *Vogel's, Textbook of Practical Organic Chemistry*, Pearson Education Limited, 1989.

34 C. S. Marvel and O. Kamm, *J. Am. Chem. Soc.*, 1919, **41**, 276.

35 J. Khazir, P. P. Singh, D. M. Reddy, I. Hyder, S. Shafi, S. D. Sawant, G. Chashoo, A. Mahajan, M. S. Alam, A. K. Saxena, S. Arvinda, B. D. Gupta and H. M. S. Kumar, *Eur. J. Med. Chem.*, 2013, **63**, 279.

36 Raunak, V. Kumar, S. Mukherjee, Poonam, A. K. Prasad, C. E. Olsen, S. J. C. Schäffer, S. K. Sharma, A. C. Watterson, W. Errington and V. S. Parmar, *Tetrahedron*, 2005, **61**, 5687.

37 S. Malhotra, S. Balwani, A. Dhawan, B. K. Singh, S. Kumar, R. Thimmulappa, S. Biswal, C. E. Olsen, E. Van der Eycken, A. K. Prasad, B. Ghosh and V. S. Parmar, *Med. Chem. Commun.*, 2011, **2**, 743.

38 J. M. Vorbrüggen, K. Kvolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234–1255.

39 R. Dalpozzo, A. De Nino, L. Maiuolo, A. Procopio, R. Romeo and G. Sindona, *Synthesis*, 2002, **2**, 172–174.

40 O. Bortolini, A. De Nino, T. Eliseo, R. Gavioli, L. Maiuolo, B. Russo and F. Sforza, *Bioorg. Med. Chem.*, 2010, **18**, 6970.

