REGIOCONTROLLED 1,3-DIPOLAR CYCLOADDITIONS OF NITRILE IMINES WITH ACETYLENES AND α,β-UNSATURATED SYSTEMS: SYNTHESIS OF POLYSUBSTITUTED AND RING FUSED PYRAZOLES WITH PHARMACEUTICAL ACTIVITY

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Abstract. *1,3-Dipolar cycloadditions of nitrile imines with functionalised acetylenes and α,β-unsaturated systems of various sizes have been studied. After cycloaddition, mono- and ring-fused pyrazoles have been obtained. Computational studies allowed a theoretical description of the observed reactivity, in agreement with the experimentally observed regio-chemistry. Selected targeted structures for medicinal applications have been synthetized and in vitro cytotoxicity showed that these pyrazoles can be considered powerful lead compounds for further medicinally relevant targets development.*

Contents

1. Introduction

Pyrazole, a five membered aromatic heterocycle containing two adjacent nitrogen atoms, is a motif found in a number of small molecules that possess a wide range of pharmaceutical and agricultural

activities.[1,2] Moreover, some pyrazoles are used in the food industry, in supramolecular and polymer chemistry, as colourings or UV stabilizers,[3] in the chemistry of complexes with phosphorescent properties[4] and as ligands for transition metal-catalyzed reactions.[5]

More in detail, a large number of top selling drugs containing pyrazoles can be found, exhibiting antispasmodic, anti-inflammatory, antibacterial, antihyperglicemic, antidepressive and, more recently, antitumor activities (Figure 1). For example, Celecoxib (**I**), a simple trisubstituted pyrazole, sold by Pfizer under the trade name of Celebrex, is used as a nonsteroidal anti-inflammatory drug;[6] Crizotinib (**II**), also sold by Pfizer, under the trade name of Xalkori, is a disubstituted simple pyrazole with antitumor properties.[7]

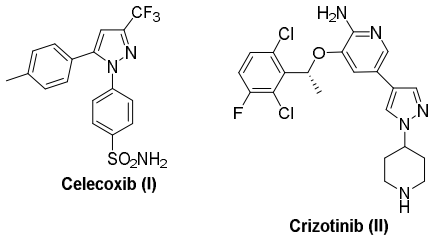


Figure 1. Celecoxib and Crizotinib: two Active Pharmaceuticals Ingredients (APIs) characterized by the pyrazole motif.

In the last decades, discovery and development of new drugs for the treatment of tumors has been a challenging feature in medicinal chemistry and drug delivery. For this purpose, pyrazolic systems are very often found as a recurrent molecular scaffold, synthesized in order to construct libraries of similar, same-core containing compounds.[3]

Ring-fused pyrazoles are, if possible, even more important than simple rings in the above mentioned applicative categories (Figure 2). An outstanding example is represented by Viagra (Sildenafil citrate, **III**), commercialized by Pfizer with high commercial success, its central core being constituted by a pyrimidinone-fused pyrazole. In addition, Apixaban (**IV**), developed by Bristol-Myers Squibb in collaboration with Pfizer, actually in phase III of clinical trials due to the excellent anticoagulant properties, contains a δ-lactamic and the a pyrazolic functionalities fused together.[8] Moreover, condensed pyrazole derivatives have often shown antiproliferative activities towards a wide number of cellular lines and are often employed in anticancer drug discovery screening.

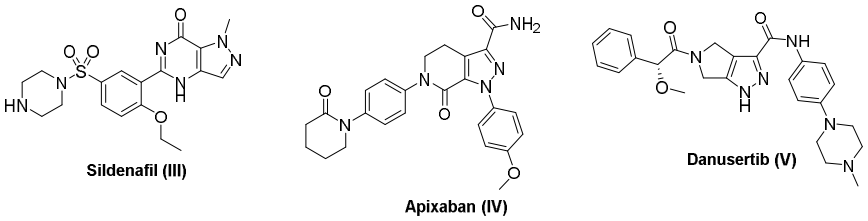
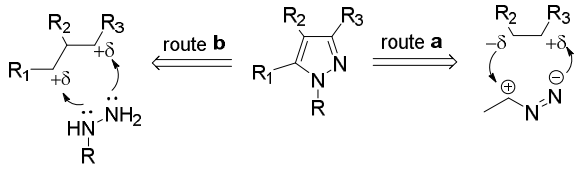


Figure 2. Ring fused pyrazoles as API in commercially available drugs.

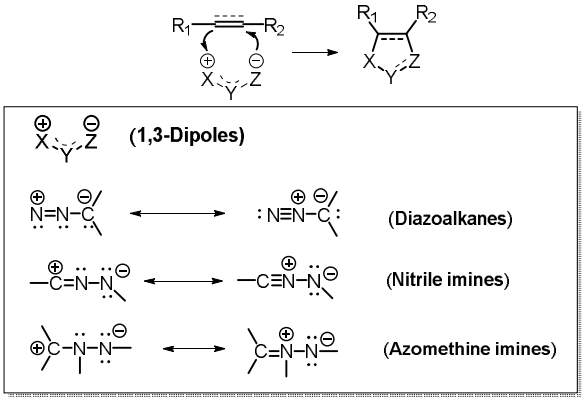
For example, pyrrolo-[3,4-c]-pyrazole **V** (Danusertib), an Aurora kinase inhibitor, has advanced in phase II clinical trials for the treatment of Bcr-Abl positive leukemias, due to the good pharmacokinetic properties along with general safety profiles shown in phase I clinical studies.[9]

Conventional approaches for the preparation of substituted pyrazoles involve either the construction of two C-N bonds by cyclocondensation of hydrazines with 1,3-dielectrophilic compounds (Scheme 1, route b) or the generation of one C-N and one C-C bond by 1,3-dipolar [3+2] cycloaddition (Scheme 1, route a).[3]



Scheme 1. General approaches for the synthesis of pyrazoles.

The 1,3-dipolar cycloaddition (1,3-DC) reaction has been employed as one of the most powerful synthetic tools to provide substituted pyrazoles. Compared to cyclocondensations between hydrazines and 1,3-dielectrophiles, 1,3-DCs are intrinsically more highly regioselective owing to the significant electronegative difference between the N and the C atom of the substrate.[3] Three main classes of 1,3-dipoles have been used as [C,N,N] synthons, namely, diazoalkanes, azomethine imines and nitrile imines, whereas the [C,C] fragment usually comes from activated π-bonds of alkanes and alkynes (Scheme 2).

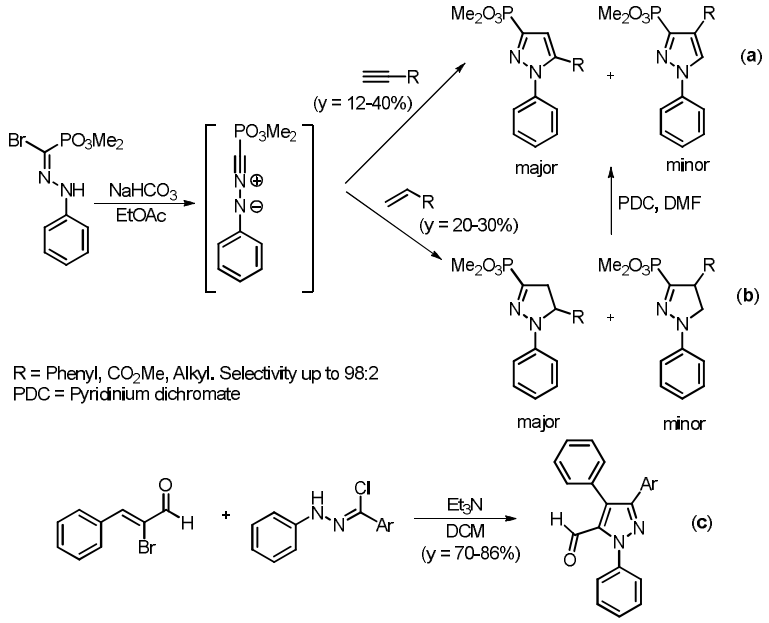


Scheme 2. Most common dipoles for 1,3-dipolar cycloadditions leading to pyrazoles.

Nitrile imines are usually generated in situ by treatment of hydrazonoyl halides with a base. Their 1,3-DC to alkynes (Scheme 3, route a)[10] or alkenes bearing a leaving group (such as α-bromo-α,β-unsaturated aldehydes in Scheme 3, route c)[11] leads directly to pyrazoles, while addition to simple activated double bonds produces pyrazolines (Scheme 3, route b)[10] that must be subsequently oxidized to the desired aromatic pyrazole.

In the present chapter regiocontrolled 1,3-DC cycloadditions of *C*-aryl-*N*-aryl and *C*-carboxymethyl-*N*-aryl nitrile imines with propiolates (see 2.1.), sulphur-based acetylenes (see 2.2.) and ynamides (see 2.3.)

leading to polyfunctionalized simple pyrazoles are presented. Moreover, their cycloaddition with cycloalkenones (see 3.1.), α,β-unsaturated lactones, thiolactones and lactams (see 3.2.) affording ring-fused pyrazoles are also described.



Scheme 3. Nitrile imine formation and cycloaddition with alkynes (a), alkenes followed by oxidation (b) and alkene bearing a leaving group (c).

The regiochemistry of such reactions was controlled by varying the experimental conditions and the substrates reactivity, developing sometimes regio-divergent approaches. The observed data were explained by means of computational methods and a rationale of the experimental reactivity and selectivity was prosed in all the cases. Moreover, to demonstrate the applicability of the developed methodologies in the field of drug design and medicinal chemistry, two polysubstituted pyrazoles (see 4.1.) and a complex molecular target characterized by a ring-fused pyrazole core (see 4.2.) were prepared and tested successfully as potential multi-kinase inhibitors towards multiple cancer cell lines.

2. 1,3-Dipolar cycloaddition of nitrile imines with acetylenes

2.1. 1,3-Dipolar cycloaddition of nitrile imines with activated acetylenes: regiocontrolled synthesis of 4- and 5-substituted pyrazoles

In contrast with the great number of 1,3-dipolar cycloadditions (1,3-DCs) of nitrile imines involving alkenes as dipolarophiles, a lack of general investigation was found about the employment of alkynes as reactive partners. Although they exhibit a slightly reduced dipolarophilic activity,[12] compared to double bonds, they still represent a powerful and convenient platform for the synthesis of substituted pyrazole rings, since an additional oxidative aromatization step is not required. Besides a couple of explorative reactions

presented by Huisgen in 1962[13] and 1967,[14] the only regioselective synthesis of pyrazoles by the present method, known at the time, was a 1,3-DC of *C*-dimethoxyphosphono *N*-aryl nitrile imines with monosubstituted acetylenes and methyl propiolate for the obtainment of 5-substituted-3-dimethoxyphosphonopyrazoles with yields in the range of 12-40%.[10]

With the aim to extend the scope of nitrile imines cycloadditions and to provide a general and regioselective method for the synthesis of 5- and 4-substituted pyrazoles, the 1,3-DC of *C*-aryl-*N*-aryl and *C*-carboxymethyl-*N*-aryl nitrile imines (derived from hydrazonoyl chlorides **2** and **1** respectively) with activated acetylenes such as benzyl propiolate **3** and *N*-phenyl-propiolamide **4** was studied.[15] The additional challenge in the employment of such compounds lay in the higher propensity to form 4-substitued pyrazoles, often in balanced regioisomeric mixtures.[14] Moreover, since a catalytic amount of Lewis acid was found to be effective in controlling the reaction outcome of 1,3-DC of certain dipoles,[16] a particularly oxophilic rare-earth-metal triflate, such as $Sc(OTf)_3$, was employed in order to gain control of the regiochemistry of the present process, an unprecedented feature in nitrile imine cycloadditions with acetylenes (Figure 3).

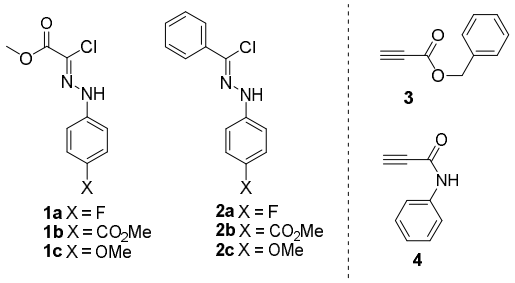


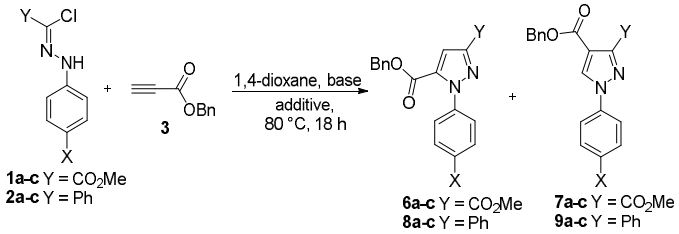
Figure 3. Dipolarophiles and hydrazonoyl chlorides employed in the present reaction.

The conditions required for the cycloaddition of **3** with **1a-c** and **2a-c** were next examined. The reactions were carried out in dry 1,4-dioxane at 80 °C for 18 h until complete conversion of **3**, employing a stoichiometric amount of hydrazonoyl chlorides **1** or **2**. The optimal base for the reaction with substrates **1a-c** was found to be Ag_2CO_3 (2.5 equivalents) while Et_3N performed better for *C*-aryl-*N*-aryl nitrile imines, derived from **2a-c**. The regioisomeric ratio was always calculated by ^1H NMR analysis of the crude mixtures (identification was carried out taking advantage of the CH signal of C-5 of the pyrazole ring which resonates at ca. 8 ppm) and the good yields (58-84%) were always referred to the sum of the two regioisomers, isolated after column chromatography. Both *C*-carboxymethyl-*N*-aryl nitrile imines derived from **1a-c** and *C*-aryl-*N*-aryl nitrile imines derived **2a-c** gave generally balanced mixtures of cycloadducts, with the exception of a slight preference in favour of 5-substituted pyrazoles in the case of products **6a/7a** (74:26) and **8b/9b** (84:16).

On the other hand, the reaction between **3** and **1a-c** conducted with a catalytic amount (10 mol%) $Sc(OTf)_3$ not only showed a general improved efficiency (88-92% yield) but resulted in a drastic regiochemistry inversion accompanied by enhanced regioselectivity in favour of 4-substitued pyrazoles, with the highest value of 9:91 obtained for products **6c**/**7c**. Nevertheless, addition of $Sc(OTf)_3$ did not affect greatly yield and regioselectivity in the cycloaddition between **3** and nitrile imines derived from **2a-c**. While a slight increase in the amount of 4-substituted products was observed in case of **8a/9a** and **8b/9b**, a deviant

result arose for cycloadducts **8c/9c**, maintaining the 5-substitued pyrazole as the major regioisomer (75:25). Moreover, in the presence of $Sc(OTf)_3$, the desired cycloaddition reaction did not take place when Ag_2CO_3 was employed as base with precursors **2a-c**, resulting only in unidentified by-products (Table 1).

Table 1. 1,3-DC of benzyl propiolate **3** with nitrile imines derived from **1a-c** and **2a-c**.

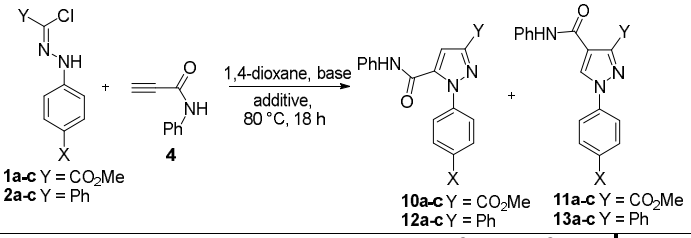


Entry	1,3-Dipole	Base	Cycloadducts	Yield (%)[a]	Ratio[a]	Yield (%)[b]	Ratio[b]
1	**1a**	Ag_2CO_3	**6a/7a**	61	74:26	90	21:79
2	**1b**	Ag_2CO_3	**6b/7b**	58	52:48	88	18:82
3	**1c**	Ag_2CO_3	**6c/7c**	84	53:47	92	9:91
4	**2a**	Et_3N	**8a/9a**	76	65:35	70	54:64
5	**2b**	Et_3N	**8b/9b**	63	84:16	65	60:40
6	**2c**	Et_3N	**8c/9c**	65	55:45	61	75:25

(a) Without $Sc(OTf)_3$. (b) With 10 mol% of $Sc(OTf)_3$

Under the same reaction conditions also dipolarophile **4** was found to be a suitable partner in the uncatalyzed cycloaddition with both *C*-carboxymethyl-*N*-aryl and *C*-aryl-*N*-aryl nitrile imines (yields ranging from 56% to 90%) giving rise to 5-substitued pyrazoles as major products with improved regioselectivity (68:32-88:12) compared to benzyl propiolate **3** (Table 2).

Table 2. 1,3-DC of *N*-benzyl propiolamide **4** with nitrile imines derived from **1a-c** and **2a-c**.



Entry	1,3-Dipole	Base	Cycloadducts	Yield (%)[a]	Ratio[a]	Yield (%)[b]	Ratio[b]
1	**1a**	Ag_2CO_3	**10a/11a**	66	83:17	94	8:92
2	**1b**	Ag_2CO_3	**10b/11b**	63	72:28	89	39:61
3	**1c**	Ag_2CO_3	**10c/11c**	90	88:12	95	32:68
4	**2a**	Et_3N	**12a/13a**	56	73:27	60	63:37
5	**2b**	Et_3N	**12b/13b**	71	68:32	68	51:49
6	**2c**	Et_3N	**12c/13c**	60	75:25	58	77:23

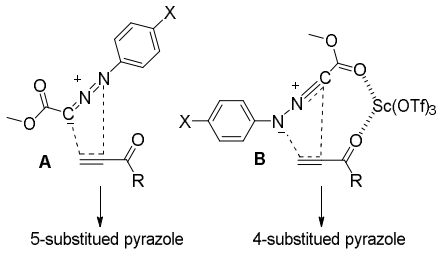
(a) Without $Sc(OTf)_3$. (b) With 10 mol% of $Sc(OTf)_3$

Again, when a catalytic amount of $Sc(OTf)_3$ was added improved efficiency and regiochemistry inversion was observed in the reaction with dipolarophiles derived from hydrazonoyl chlorides **1a-c** (94%

yield and 8:92 ratio as the leading result for pyrazoles **10a/11a**). On the other hand, the reaction involving **2a-c** remained quite unaffected and failed when Ag_2CO_3 was used as base.

To tentatively explain the global regiochemical outcomes, the electronic characteristics of the two substrates were deeply analysed. First of all, taking advantage of the global electrophilicity index ω proposed by Parr,[17] the present reaction was classified as a normal electron-demand (NED) cycloaddition. This reaction follows a polar mechanism, with the charge transfer occurring from the dipole (typical value of ω is around 0.28, i.e. marginal electrophile that can act as nucleophile) to the dipolarophile (ω = 1.52 for **3**, i.e. strong electrophile) leading to 5-substitued pyrazoles mainly (**A**). Similar data were reported for the cycloaddition of *N*-oxides with electron-poor acetylenes, showing comparable regiochemistry outcomes (regioisomeric mixtures with 5-substituted isoxazoles as major products); computational studies also confirmed the experimental results.[18]

On the other hand, the reversal regioselectivity observed in the 1,3-DC of **3** or **4** and nitrile imines derived from **1a-c** under $Sc(OTf)_3$ catalysis might explained invoking a chelate transition state (**B**), where the carboxymethyl of the dipole and the carboxyl groups of the dipolarophiles coordinate the $Sc(OTf)_3$ in a bidentate fashion. This also explains the improved yield observed, since the high affinity of scandium for the oxygen of the carboxymethyl group on the dipole enhances the electrophilicity of the C atom, resulting in a more reactive species. All these hypothesis seem to be strengthened through the relatively poor effectiveness of this type of catalysis on substrates derived from **2a-c**, in which, coordination of $Sc(OTf)_3$ to the nitrile imine cannot occur. Again, similar reaction outcomes were observed in the 1,3-DC of nitrones with α,β-unsaturated compounds (Scheme 4).



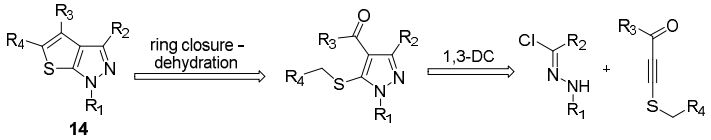
Scheme 4. Hypothesized interaction mode leading to 5- or 4-substituted pyrazoles.

2.2. 1,3-Dipolar Cycloaddition with sulphur-based acetylenes: regiocontrolled synthesis of thieno[2,3-*c*]pyrazoles

Heteroatom-substituted acetylenes have been scarcely studied as dipolarophiles in 1,3-DCs with nitrile imines. More specifically, as sulphur-based acetylenes may be concerned: while alkynyl phenyl sulphides have never been employed in this kind of 1,3-DC, just one example existed about alkynyl phenyl sulphones, which were reported to add to *N*-phenyl-*C*-phenyl nitrile imines to form 4-substitued pyrazoles almost exclusively.[19] Nevertheless, these kinds of dipolarophiles, and the possibility of controlling the regiochemistry in their 1,3-DC with different nitrile imines remained quite unexplored. Although, the present synthetic methodology could play an important role in the installation of the *S* atom in the right

position of the pyrazole ring allowing, after synthetic elaborations, the achievement of ring-fused thienopyrazoles of type **14**.[20]

This molecular motif represents an important class of potent kinase inhibitors, object of a great number of synthetic efforts: either starting from functionalized pyrazoles, fused with the thiophene ring at the end of the synthetic sequence, or with a suitable thiophene, fused with the pyrazole ring in the final step.[21] Nevertheless, the construction of the thiophene ring for intramolecular cyclization of a conveniently synthesized pyrazole (Scheme 5) was an unprecedented, yet quite appealing method for the synthesis of such ring-fused systems.



Scheme 5. Retrosynthetic analysis towards thieno[2,3-c]pyrazoles.

With the aim to extend the generality of the 1,3-DC of alkynyl phenyl sulphones with nitrile imines, reporting for the first time their 1,3-DC with *C*-carboxymethyl-*N*-aryl nitrile imine and to investigate the reactivity of previously unreported alkynyl phenyl sulphides, both terminal (**15** and **17**) and disubstituted (**16** and **18**) acetylenes were engaged in 1,3-DC reactions with three nitrile imines derived from hydrazonoyl chlorides **19a-c** (Figure 4). All these substrates were readily prepared according to literature procedures.

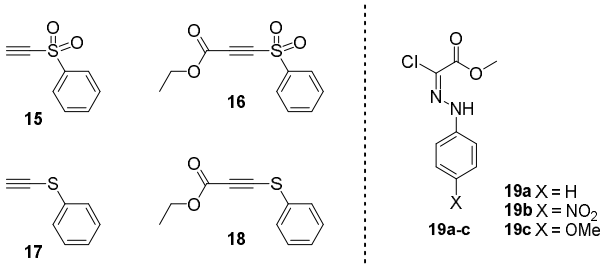


Figure 4. Dipolarophiles and hydrazonoyl chlorides employed in the present reaction.

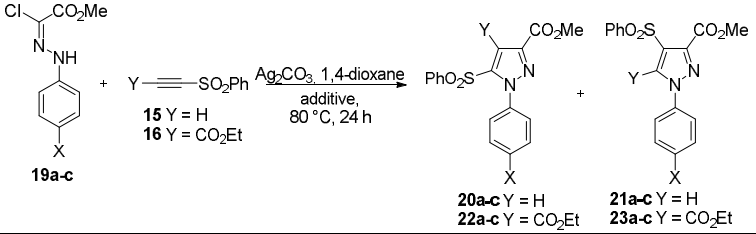
The conditions required for the cycloaddition of alkynyl phenyl sulphones **15** and **16** with *C*-carboxymethyl-*N*-aryl nitrile imines derived from **19a-c** were next examined. The reactions were carried out in dry 1,4-dioxane at 80 °C for 24 h, employing a stoichiometric amount of hydrazonoyl chlorides **19a-c** and using Ag_2CO_3 (2.5 equivalents) as base; the same reactions were then repeated, in the presence of a catalytic amount of $Sc(OTf)_3$.

The 1,3-DC of monosubstituted acetylene **15** gave quite balanced mixtures of regioisomers (**20a-c**/**21a-c**) in moderate to good yields (always referred as the sum of regioisomers) ranging from 35% to 58%, as the uncatalyzed reaction is concerned. On the other hand, upon addition of the Lewis acid, the regioisomeric ratio improved greatly in favour of the 4-isomer (12:88 ratio as the leading result for pyrazoles **20a**/**21a**), while the efficiency slightly decreased (34-39% yield). These results are in accordance both with

the data obtained by Zecchi[18] with *C*-aryl-*N*-aryl nitrile imines and with our previous work involving dipolarophiles **3** and **4** (see 2.1.). The 1,3-DC is taking place as a NED-cycloaddition with a HOMO-(dipole) LUMO-(dipolarophile) interaction, due to the large LUMO coefficient on the β-carbon of the triple bond, induced by the strong electron-withdrawing effect of the sulphonyl group. Again, upon Sc-catalysis a chelate transition state (between the SO_2Ph and the CO_2Me groups) may be invoked to explain the regiochemical outcome.

Disubstituted acetylene **16** gave good yields (60-65%) of cycloadducts **22a-c**/**23a-c** with higher quantities of the 4-isomer in the uncatalyzed process, but in this case the reaction was quite unaffected by the addition of $Sc(OTf)_3$ (slightly increased efficiency and regioselectivity: 8:92 ratio and 72% yield against 11:89 ratio and 63% yield for pyrazoles **22a/23a** may be taken as representative data). Therefore, we believe that this kind of 1,3-DC is substrate-controlled and does not take place under the direction of the Lewis acid catalyst (Table 3).

Table 3. 1,3-DC of alkynl phenyl sulphones **15** and **16** with nitrile imines derived from **19a-c**.



Entry	1,3-Dipole	Dipolarophile	Cycloadducts	Yield (%)[a]	Ratio[a]	Yield (%)[b]	Ratio[b]
1	**19a**	**15**	**20a/21a**	49	35:65	38	12:88
2	**19b**	**15**	**20b/21b**	35	40:60	34	14:86
3	**19c**	**15**	**20c/21c**	58	54:46	39	14:86
4	**19a**	**16**	**22a/23a**	63	11:89	72	8:92
5	**19b**	**16**	**22b/23b**	65	20:80	67	15:85
6	**19c**	**16**	**22c/23c**	60	10:90	64	9:91

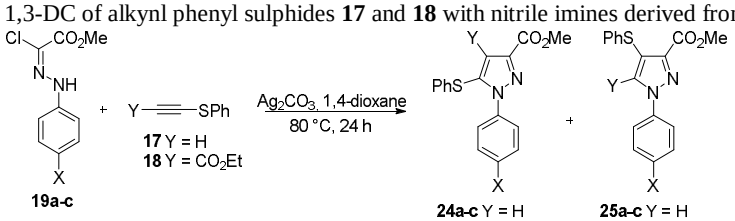
(a) Without $Sc(OTf)_3$. (b) With 10 mol% of $Sc(OTf)_3$

We next examined the 1,3-DC of alkynyl phenyl sulphides **17** and **18** with the nitrile imines derived from **19a-c** (Table 4). A reversal in the regiochemistry was observed in favour of the 5-substitued pyrazole, which was always obtained in good yields (45-70%) as a single product, with or without $Sc(OTf)_3$. These results are in accordance with the data obtained for the 1,3-DC of nitrile imines or nitrile *N*-oxides with electron-rich acetylenes,[14,18] which tend to be much more regioselective with respect of electron-poor ones (such as **3**, **4**, **15** or **16**), with the almost exclusive formation of 5-substituted pyrazoles. The reaction is now taking place as an inverse electron-demand (IED) cycloaddition with a LUMO-(dipole) HOMO-(dipolarophile) interaction, due to the large HOMO coefficient on the β-carbon of the triple bond, induced by the strong electron-donating effect of the sulphide group.

Regioisomeric ratios were calculated by ^1H NMR analysis on the crude reaction mixtures. While in the case of products **20a-c**/**21a-c** and **24a-c**/**25a-c** identification of the two regioisomers was carried out by ^1H NMR analysis (taking advantage of the fact that it is known that the CH signal of C-5 of the pyrazole ring

resonates at around 8 ppm), NOE experiments were carried out in case the of fully substituted cycloadducts **22a-c**/**23a-c** and **26a-c** to identify the correct structure.

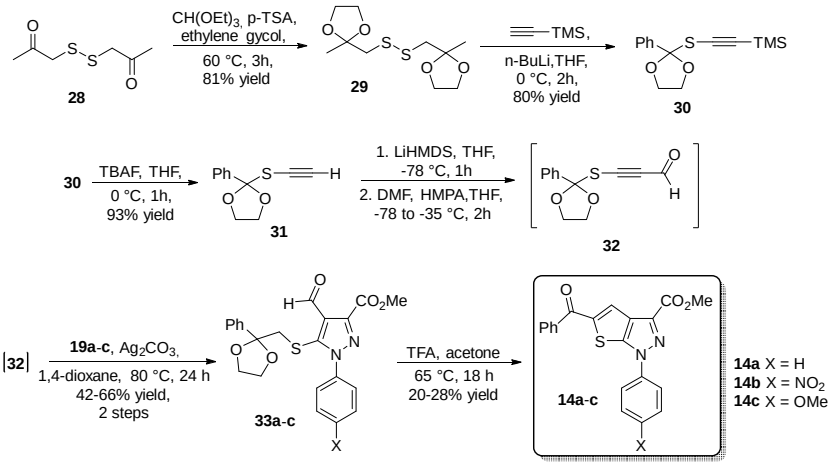
Table 4. 1,3-DC of alkynl phenyl sulphides **17** and **18** with nitrile imines derived from **19a-c**.



Entry	1,3-Dipole	Dipolarophile	Cycloadducts	Yield (%)	Ratio
1	**19a**	**17**	**24a/25a**	70	>99:1
2	**19b**	**17**	**24b/25b**	57	>99:1
3	**19c**	**17**	**24c/25c**	67	>99:1
4	**19a**	**18**	**26a/27a**	50	>99:1
5	**19b**	**18**	**26b/27b**	45	>99:1
6	**19c**	**18**	**26c/27c**	67	>99:1

With these results in hand, we were able to show that different S-based functionalities on the dipolarophile control the regiochemistry of the 1,3-DC: in particular, the synergistic regiochemical effect shown by the electron-releasing properties of the sulphide group, along with the electron-withdrawing ones of the ester (dipolarophile **18**), led to the formation of only one regioisomer (pyrazoles **26a-c**). This regiochemistry is correct for the preparation of ring-fuse thienopyrazoles of type **14**. To enable this, we designed an analogue of dipole **18** in which the triple bond bears a masked S-phenacyl substituent and a formyl moiety. This new designed substrate maintains the same electronic properties of its analogue **18**, being at the same time amiable of intramolecular aldol condensation with concomitant aromatization to the thiophene ring.

Therefore, the starting material was diphenacyl disulphide **28**, easily obtained through a modified literature procedure from phenacyl mercaptan (Scheme 6). After protection of **28** as dioxolane **29**, acetylene **31** was obtained by reaction with lithium trimethyl-silyl acetylene (product **30**) and subsequent deprotection with TBAF, in 60% overall yield over three steps. The abovementioned analogue of dipolarophile **18** (disubstituted acetylene **32**) was prepared by lithiation of **31** followed by the addition of a mixture of DMF-HMPA as a formylating agent. This highly reactive substrate was not isolated but immediately subjected to the standard 1,3-DC reaction conditions with **19a-c**, to yield pyrazoles **33a** in 66% yield, **33b** in 42% yield and **33c** in 43% yield. These cycloadducts were isolated as single regioisomers and fully characterized. Finally, deprotection of dioxolanes and intramolecular aldol condensation took place in a one-pot reaction, promoted by TFA. Thieno[2,3-*c*]pyrazoles **14a-c** were thus obtained in 20-28% overall yield.



Scheme 6. Synthesis of thieno[2,3-c]pyrazoles **14a-c**.

2.3. 1,3-Dipolar cycloaddition with ynamide *tert*-butyl *N*-ethynyl-*N*-phenylcarbamate: experimental and theoretical investigation

Ynamides[22] are electron rich, activated acetylenes, commonly employed in the regioselective synthesis of amino-substituted heterocycles. Their 1,3-DC with azides,[23] nitrones,[24] nitrile oxides[25] and diazoacetates[25b] have been widely investigated. Surprisingly, their reaction with nitrile imines, leading hypothetically to amino-pyrazoles, was never reported at the time.[26]

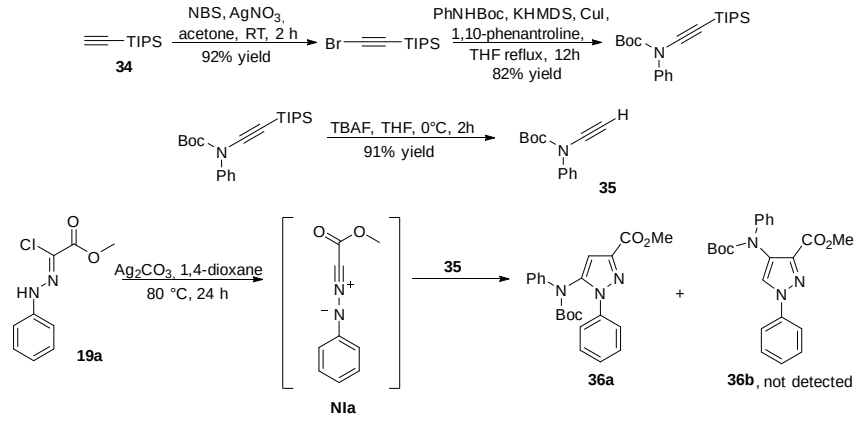
The required dipolarophile, *tert*-butyl *N*-ethynyl-*N*-phenylcarbamate **35**, was synthesized starting from triisopropylsilyl-acetylene **34**, through bromination, Cu^{I} catalysed coupling with *N*-Boc aniline[27] and protodesilylation (Scheme 7). This was engaged in the cycloaddition with *N*-phenyl-*C*-carboxymethyl nitrile imine (**NIa**), derived from **19a**, under our previously reported optimal reaction conditions: Ag_2CO_3 as the base for the generation of the 1,3-dipole from the hydrazonoyl chloride and 1,4-dioxane as the solvent, at 80 °C. The expected product was thus recovered in 43% yield after column chromatography as a single regioisomer (**37a**), which structure was confirmed by X-ray analysis.

To understand the complete regioselectivity shown by this previously unexplored 1,3-DC, prompted us to analyse its regiochemical pathway by theoretical methods. Two different models were taken into account to explain the experimental data: one, recently proposed by Houk, based on the energetic requirement needed to distort both the 1,3-dipole and the dipolarophile to the geometry of the transition states,[28] and one based on the Frontier Molecular Orbital approach (FMO).

As the first one is concerned, the most stable conformation for **35**, **NIa**, **36a**, and **36b** was found by molecular mechanics methods and the ground state structure optimized with inclusion of the solvent. A simplified model, in which the *t*-Bu group was substituted with a methyl group, was adopted for **NIa**, **36a** and **36b**. As the relative energy of the two regioisomers **36a** and **36b** were found to be very similar, thermodynamic control could not be invoked.

At this point, from the two ground states of the products, two minimum energy transition states, leading to the regioisomeric adducts (**TSa** leading to **36a** and **TSb** leading to **36b**) were calculated. **TSa** was

found to be largely asynchronous (as the C-C bond is formed well before the C-N bond) and 10 kcal/mol lower in energy than **TSb** which, in contrast, seemed to be synchronous.



Scheme 7. Synthesis of ynamide **35** and its cycloaddition with the nitrile imine derived from **19a**.

This is in perfect accordance with the complete regioselectivity showed by the reaction. A stepwise mechanism was also taken into account: if an alternative to **TSb** could not be found, the stepwise TS leading to **36a** (**TSa'**) was calculated to be almost isoergonic with **TSa**. However, intrinsic reaction coordinate calculations involving **TSa** and **TSb** showed the conversion of **35** and **NIa** into **36a** or **36b** without any intermediates, while the calculations predicted that **TSa'** did not provide the expected pyrazole **36a** as the product. Therefore, the concerted reaction pathway was taken as the correct one (Figure 5).

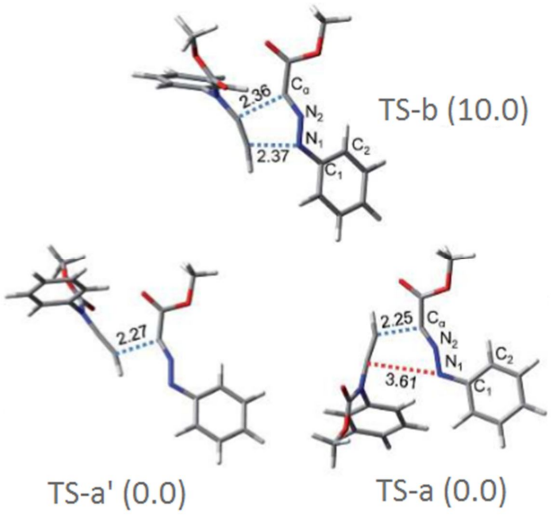


Figure 5. Calculated transition states.

Following the approach described by Houk,[28] the energies contributing to the transition states (interaction and distortion energies) were then calculated. As it is possible to see in Table 5, the interaction energies for **TSa** and **TSb** are almost the same, while the distortion energies largely favour **TSa**, especially as the dipole counterpart is concerned. This suggests that the source of the reaction selectivity is the different energy required to change the reactants to the correct geometries for the transition states.

Table 5. Calculated energies contributing to the transition states.

TS	$\Delta E_{ynamide}$	ΔE_{dipole}	$\Delta E_{interaction}$	ΔE_{tot}
TSa	+2.13	+7.22	-6.37	+2.98
TSb	+7.47	+13.40	-7.60	+13.27

As the second approach is concerned, the FMO analysis showed that the smaller energy gap (3.8 eV) for **TSa** was between the HOMO of the dipolarophile and the LUMO of the dipole (classifying the present reaction as an IED 1,3-dipolar cycloaddition). On the contrary, the smaller gap for **TSb** was found to be between the HOMO of the dipole and the LUMO of the dipolarophile, confirming that the distortion energy is the reason of the regioselectivity of the process (Figure 6).

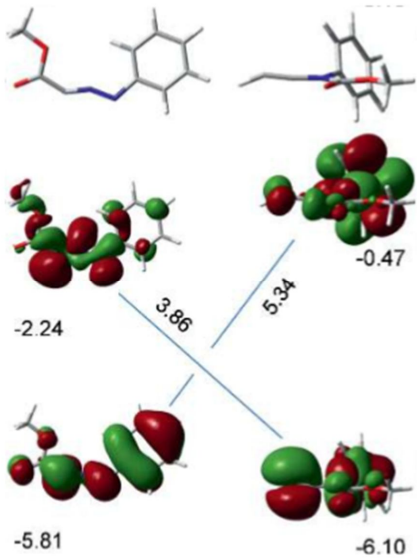
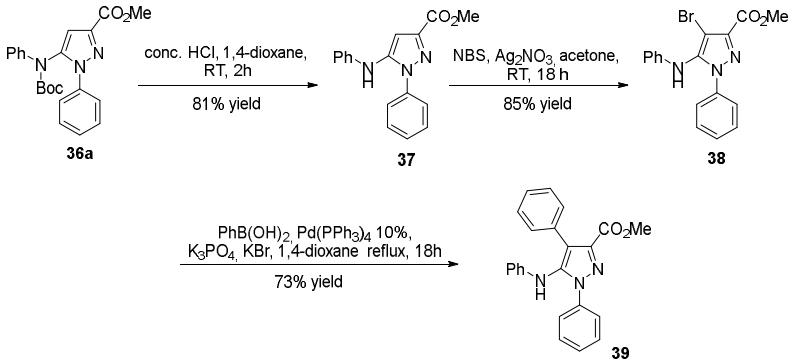


Figure 6. HOMO-LUMO interaction.

The regiochemical results obtained for this cycloaddition are in perfect agreement with the literature and with our previously described reaction of sulphur-based acetylenes with nitrile imines (see 2.2.). Dipolarophile **35** may in fact be considered an electron rich acetylene, such as **17** or **18** with the tendency to render 5-substituted pyrazoles with complete regioselectivity.

To demonstrate the synthetic utility of the described cycloaddition, the obtained product **36a** was proved to undergo easily activation and functionalization of the C-4, rendering this strategy prone to the

preparation of fully substituted 5-aminopyrazoles. After Boc group cleavage, unprotected 5-aminopyrazole **37** underwent bromination at the C-4 by reaction with NBS (*N*-bromosuccinimide) and $AgNO_3$ in acetone to obtain product **38**. Hereafter, Suzuki coupling with phenylboronic acid under standard reaction conditions[29] afforded tetrasubstituted pyrazole **39** in high yield. Such type of functionalization was unprecedented, as Suzuki couplings were never attempted on free (or protected) aminopyrazoles as the heteroaryl halide counterpart (Scheme 8).[29,30]



Scheme 8. Preparation of fully substituted amino-pyrazole **39** through bromination-Suzuki coupling.

3. 1,3-Dipolar cycloaddition of nitrile imines with cyclic dipolarophiles

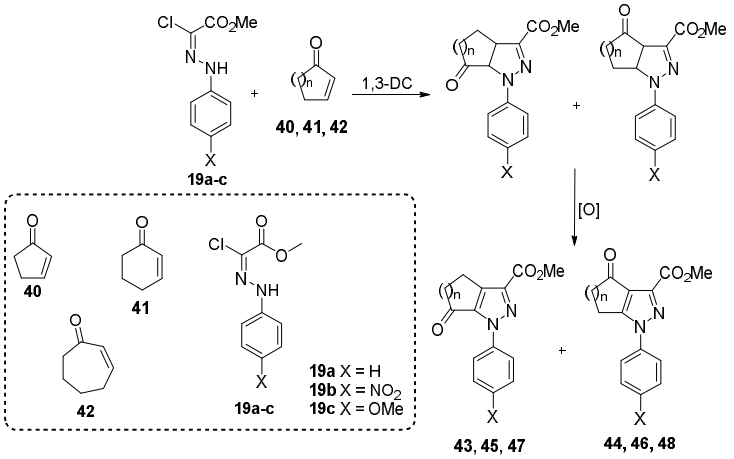
3.1. 1,3-Dipolar cycloaddition of nitrile imines with α,β-unsaturated ketones: regiocontrolled synthesis of ring-fused pyrazoles

The preparation of pyrazole-fused ring derivatives seems to be very important and challenging from the synthetic point of view.[31] One of the most straightforward strategies for the synthesis of ring-fused 5 membered heterocycles is the direct cycloaddition of nitrile imines with α,β-unsaturated dipolarophiles. However, few papers existed relying on such methodology. If the reactivity of some dipoles, such as azomethine imines[32] and ylides[33] were explored in the 1,3-DC with cyclic enones leading to various heterocycles, as the preparation of pyrazoles is concerned, only one report described the reaction of diphenylnitrile imine with cycloalkenones, leading to balanced regioisomeric mixtures of ring-fused pyrazoles.[34]

In the presented strategy,[35] three different *C*-carboxymethyl-*N*-aryl nitrile imines derived from hydrazonoyl halides **19a**, **19b** and **19c** were reacted with cyclopentenone **40**, cyclohexenone **41** and cycloheptenone **42**. All the reaction were carried out both with Ag_2CO_3 and Et_3N as base necessary for promoting the formation of the nitrile imines, and either in the presence or absence of a catalytic amount of $Sc(OTf)_3$. The direct cycloaddition rendered regioisomeric pyrazolines, which were converted by oxidative aromatization in the desired pyrazoles (5-acyl derivatives **43** and 4-acyl derivatives **44**) in a two-step, one-pot procedure (Scheme 9).

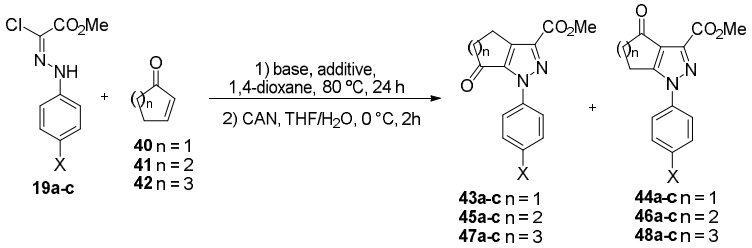
All the reactions were carried out in dry 1,4-dioxane at 80 °C for 18 h, while the aromatization step was accomplished employing CAN (cerium ammonium nitrate) as the oxidant in a 4:1 THF/H_2O mixture at 0 °C for 2 h. The results obtained are summarized in Table 6 (the structure of pyrazoles **43**-**48**, previously

characterized by NMR spectroscopy, was determined by X-rays diffraction analysis). Some general features of this reaction may therefore be observed.



Scheme 9. Synthetic strategy adopted; dipolarophiles and hydrazonoyl chlorides employed.

Table 6. 1,3-DC of enones **40**-**42** with nitrile imines derived from **19a-c**.



Entry	1,3-Dipole	Enone	Cycloadducts	Yield[a]|Yield[b] (%)	Ratio[a]|Ratio[b]	Yield[c]|Yield[d] (%)	Ratio[c]|Ratio[d]
1	**19a**	**40**	**43a/44a**	32 | 14	78:22 | 76:24	37 | 43	62:38 | 70:30
2	**19b**	**40**	**43b/44b**	-	-	-	-
3	**19c**	**40**	**43c/44c**	49 | 26	77:23 | 83:17	43 | 65	**19:81 | 20:80**
4	**19a**	**41**	**45a/46a**	42 | 19	82:18 | 99:1	72 | 60	99:1 | 99:1
5	**19b**	**41**	**45b/46b**	17 | 11	60:40 | 99:1	-	-
6	**19c**	**41**	**45c/46c**	65 | 34	80:20 | 99:1	88 | 62	90:10 | 99:1
7	**19a**	**42**	**47a/48a**	36 | 28	99:1 | 99:1	82 | 64	86:14 | 83:17
8	**19b**	**42**	**47b/48b**	-	-	-	-
9	**19c**	**42**	**47c/48c**	53 | 38	99:1 | 99:1	63 | 69	63:27 | 79:21

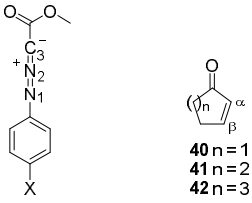
(a) With Ag_2CO_3, without $Sc(OTf)_3$. (b) With Ag_2CO_3, with 10 mol% of $Sc(OTf)_3$. (c) With Et_3N, without $Sc(OTf)_3$. (d) With Et_3N, with 10 mol% $Sc(OTf)_3$

1) The reactivity of *C*-carboxymethyl-*N*-*p*-NO_2-phenyl nitrile imine, derived from **19b** was found to be very low, affording only in one case (reaction with **41** only when Ag_2CO_3 was employed) products **45b**/**46b** in very low yield (11-17%).

2) On the other hand, *C*-carboxymethyl-*N*-*p*-MeO-phenyl nitrile imine, derived from **19c** proved to be much more reactive than its unsubstituted analogue deriving from **19a**, achieving better yields with all the dipolarophiles.
3) As the base is concerned, Et_3N usually rendered better yields than Ag_2CO_3 when **41** or **42** were employed (88% yield for products **45c**/**46c** was the best result). The lower yields observed for **40** were attributed to a competitive tendency towards enolization.
4) As the size of the dipolarophiles is concerned, enones **41** and **43** were found to be much more regioselective for the formation of 5-acyl derivatives **46** and **48** respectively. Dipolarophile **40**, on the other hand, produced a deviant result in the reaction with **19c** when Et_3N was employed giving products **43c**/**44c** in 20:80 ratio, thus favouring the 4-acyl derivative.
5) $Sc(OTf)_3$ usually improved the regioselectivity towards the 5-acyl pyrazole with Ag_2CO_3 as base, although with diminished yields. Its effect was however less important in the reaction promoted by Et_3N.

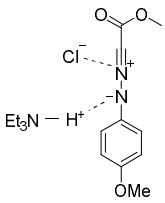
To explain some of the previously mentioned experimental features, analysis of the global and local reactivity indexes at the ground state of reagents was undertaken, through DFT calculations. First of all the chemical potential μ (Table 7) was evaluated for all the dipoles (**NIa** derived from **19a**, **NIb** derived from **19b** and **NIc** derived from **19c**) and the dipolarophiles **40**, **41** and **42**. The chemical potential for **NIa** and **NIc** was found to be higher than those for the dipolarophiles. This means that the charge transfer in the reaction takes place from the dipoles (acting as nucleophiles) to the dipolarophiles (acting as electrophiles) in a NED 1,3-DC. On the other hand, **NIb** showed a higher value of chemical potential than the dipolarophiles, and would therefore act as the electrophile in an IED cycloaddition. Thus, given the poor nucleophilicity of enones **40**, **41** and **42**, the low reactivity observed in this case for this nitrile imine may be explained.

Table 7. Chemical potential and Fukui functions contributions for **NIa-c**, **NI'c** and enones **40-42**.



NIa X = H
NIb X = NO_2
NIc X = OMe

40 n = 1
41 n = 2
42 n = 3



NI'c

Compound	μ [a.u.]	$f_k^-(N_1)$	$f_k^-(N_2)$	$f_k^-(C_3)$	Compound	μ [a.u.]	$f_k^+(C_\alpha)$	$f_k^+(C_\beta)$
NIa	-0.1399	0.14	0.00	**0.18**	**40**	-0.1409	0.10	**0.24**
NIb	-0.1693	-	-	-	**41**	-0.1423	0.07	**0.20**
NIc	-0.1293	0.12	0.00	**0.14**	**42**	-0.1429	0.04	**0.20**
NI'c	-0.0874	**0.11**	0.00	0.12				

Following an approach developed for Diels-Alder reactions,[36] and extended by Domingo et al. to 1,3-DCs,[37] the observed regioselectivity was explained as follows. Calculation of the Fukui functions contributions (f_k) to the respective atomic centres was undertaken: electrophilic, f_k^+ (for the electrophile, in this case the dipolarophiles) and nucleophilic, f_k^- (for the nucleophiles, in this case the dipoles). In this

approach, the atom of the electrophile sharing the highest f_k^+ value will react with atom of the nucleophile sharing the highest f_k^- value. As it is possible to see in Table 7 the calculations predicted that the C_3 of **NIa** and **NIc** would interact with the β carbon of the enones giving rise to the predominantly observed 5-acyl derivatives **43**, **45** and **47**.

The deviant result given by the cycloaddition of **40** with **NIc** in the presence of Et_3N (predominance of the 4-acyl pyrazole **44c**) was explained postulating that the reactive species would not be **NIc** itself, but intermediate **NI'c**, which is formed prior to the proper nitrile imine generation This takes into account that only with Et_3N (and not with Ag_2CO_3) the inversion in regioselectivity was observed. As **NI'c** is believed to be a short-living intermediate, its cycloaddition, instead of the properly formed nitrile imine, is considered to be possible only with a highly reactive dipole, as the one generated in this case form **19c**. This explains why with **19a** the regiochemical outcome was different, as in this case the reactive species was **NIa**. Finally, since the reactive site of **NI'c** is encumbered by the large groups of Et_3N, only a small dipolarophile would be able to interact successfully. This explains why only in the case of dipolarophile **40** this inversion was observed; in all other cases the enones were too large to react with **NI'c** and the species taking part to the cycloaddition was **NIc** itself (Figure 7).

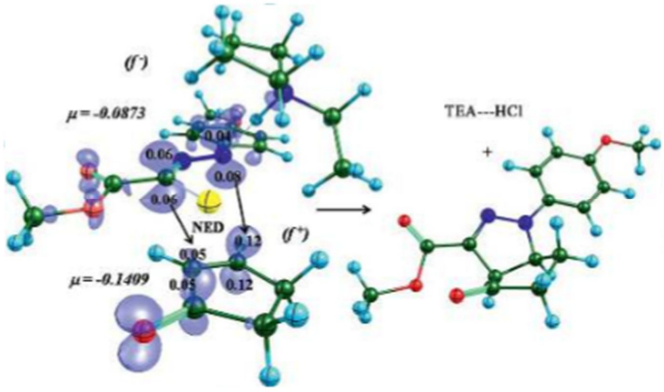


Figure 7. Interaction of **NI'c** with **40** with basins of the topologically evaluated Fukui Functions contributions.

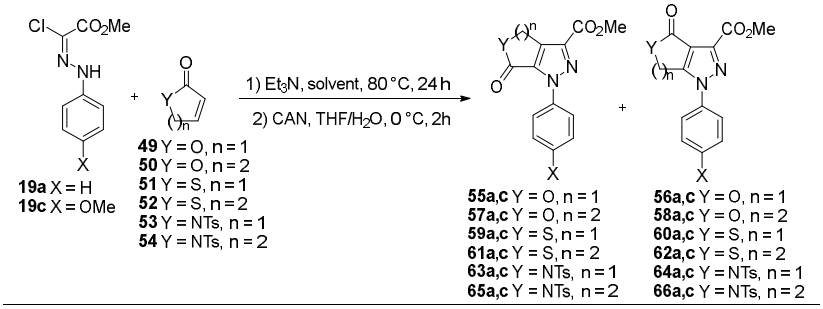
Also in this example, the chemical potential of the dipole was found to be lower than the dipolarophile, classifying the reactions involving **NI'c** as NED 1,3-DCs. Unfortunately, as the Fukui functions parameters are concerned, the calculations were in disagreement with the observed regiochemical results. However, if the Fukui functions are evaluated topologically and basins are obtained around its maximum, it can be seen that N_1 presents two asymmetrical basins. While the less reactive one (f_k^- = 0.04) is shielded by Et_3N, the other (f_k^- = 0.08) is free and prone to nucleophilic attack. Therefore, on the reactive face of **NI'c**, the largest value of f_k^- is held by N_1, in agreement with the experimental results.

3.2. 1,3-Dipolar cycloaddition of nitrile imines with α,β-unsaturated lactones, thiolactones and lactams: regiocontrolled synthesis of ring-fused pyrazoles

With the aim of expanding the scope of these kind of 1,3-DCs of nitrile imines, we moved to the evaluation of α,β-unsaturated lactones, thiolactones and lactams as dipolarophiles for the synthesis of ring-fused pyrazoles.[38] While thiolactones were yet unexplored as dipolarophiles in 1,3-DCs with nitrile imines, only coumarine derivatives, as the lactones are concerned, were involved in such reactions.[39] A six-membered α,β-unsaturated lactam bearing a good leaving group such as chlorine or morpholine, useful for aromatization, was successfully employed in an 1,3-DC with a suitable nitrile imine, for the industrial synthesis of the anticoagulant Apixaban.[8] However, a general strategy involving simple, unsubstituted cyclic acyl systems was yet unexplored.

In the present approach, the reaction of α,β-unsaturated lactones **49**, **50**, thiolactones **51**, **52** and lactams **53**, **54** with the nitrile imines derived from hydrazonoyl chlorides **19a** and **19c** were performed in dry 1,4-dioxane, employing Et_3N as base for the generation of the dipoles, at 80 °C for 18 h. In the case of the lactams, toluene as the solvent was employed in order to achieve higher yields. The aromatization step was then accomplished by oxidation of the so formed intermediate pyrazolines with CAN in a THF/H_2O mixture at 0 °C for 2h. The desired pyrazoles (5-acyl derivatives **55**, **57**, **59**, **61**, **63**, **65** and 4-acyl derivatives **56**, **58**, **60**, **62**, **64**, **66**) were therefore obtained in a two-step, one-pot procedure. The results are summarized in Table 8 (the structure of pyrazoles **55-66**, previously characterized by NMR spectroscopy, was determined by X-rays diffraction analysis).

Table 8. Cycloaddition of α,β-unsaturated cyclic acyl systems **49-54** with the nitrile imines derived from **19a,c**.



Entry	1,3-Dipole	Dipolarophile	Cycloadducts	Yield (%)	Ratio
1	**19a**	**49**	**55a/56a**	14	70:30
2	**19c**	**49**	**55c/56c**	31	**14:86**
3	**19a**	**50**	**57a/58a**	47	52:48
4	**19c**	**50**	**57c/58c**	66	87:13
5	**19a**	**51**	**59a/60a**	21	99:1
6	**19c**	**51**	**59c/60c**	36	**82:18**
7	**19a**	**52**	**61a/62a**	56	81:19
8	**19c**	**52**	**61c/62c**	77	86:14
9	**19a**	**53**	**63a/64a**	38	72:28
10	**19c**	**53**	**63c/64c**	35	61:39
11	**19a**	**54**	**65a/66a**	74	83:17
12	**19c**	**54**	**65c/66c**	68	83:17

It appeared from Table 8 that the nitrile imine from **19a** gave a large prevalence of the 5-acyl derivative with five-membered lactone **49** (entry 1), thiolactone **51** (entry 5) lactam **53** (entry 9) and with six-membered thiolactone **52** (entry 7) and lactam **54** (entry 11). A balanced mixture of 5- and 4-acyl derivatives has been obtained with six-membered lactone **50** (entry 3). The nitrile imine from **19c** gave also the 5-acyl derivative as the major product (entries 4, 6, 8, 10, 12) but rendered a deviant result, with inversion of regiochemistry, with the five-membered lactone **49** (entry 2). The regioisomeric ratio is therefore influenced by the X substituent at the *para* position of the phenyl ring and by the nature of the nitrile imine.

Similarly, to the previously reported cycloadditions of cyclic enones with nitrile imines, to explain some of the previously mentioned experimental features, analysis of the global and local reactivity indexes at the ground state of reagents was undertaken, through DFT calculations. Also in this case the chemical potential μ of **NIa** (derived from **19a**) and **NIc** (derived from **19c**) was found to be higher than those for the dipolarophiles. This means that the charge transfer in the reaction takes place from the dipoles (acting as nucleophiles) to the dipolarophiles (acting as electrophiles) in a NED 1,3-DC.

Again, calculation of the Fukui functions contributions (f_k) to the respective atomic centres predicted that the C_3 of **NIa** and **NIc** would interact with the β carbon of the unsaturated cyclic systems giving rise to the predominantly observed 5-acyl derivatives. Therefore, if the reactive species in the cycloaddition step is the nitrile imine itself the 5-acyl pyrazole will be the major product. This means that in case of **19c** with **49** the reactive species is not **NIc** but the previously postulated **NI'c** intermediate. Once again, the use of topological analysis of the Fukui functions allows a theoretical description of the local reactivity in agreement with the experimentally observed regiochemistry.

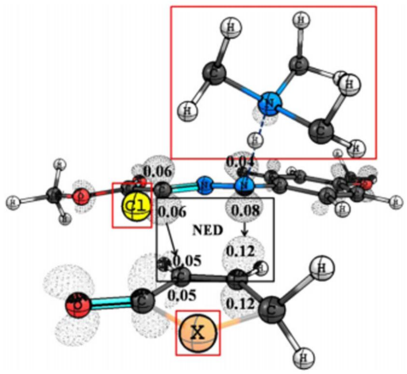


Figure 8. Interaction of **NI'c** with **49** (X = O), **51** (X = S) or **40** (X = CH_2) with basins of the topologically evaluated Fukui functions contributions.

However, the previous statement, applied to the reaction of **51** with **19c**, implies that the reactive species is not **NI'c** but **NIc** itself (predominance of the 5-acyl pyrazole **59c** over **60c**). If all the other dipolarophiles are clearly too large to interact with the intermediate (as stated in the previous chapter), **51** is comparatively as small as **49** (or the previously employed **40**) and should therefore successfully react with **NI'c** to give predominantly **60c**. To explain this new deviant result, we have proposed that the electrostatic

repulsive interaction with the *S*-atom of **51** with the *Cl*-atom of **NI'c** would be larger than the ones with the O-atom of **49**. This argumentation relies on the larger size of the S compared to O, resulting in a more polarizable surrounding density with higher repulsive interactions. This was confirmed using a calculation methodology, which enables to visualize the electronic charge density excess or deficiency around the reactive regions of the molecules. This method combines electronic charge density and molecular electrostatic potential values (MEP), as shown in Figure 9.[40]

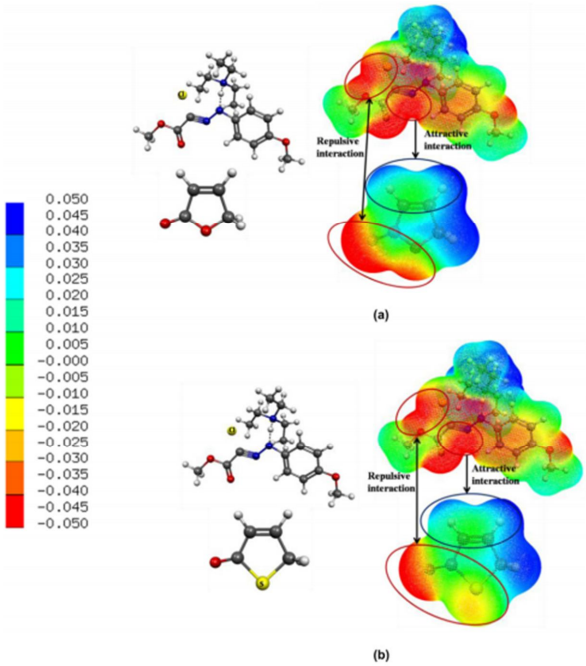


Figure 9. Electrostatic interaction analysis between **NI'c** and **49** or **51**.

4. Pyrazole derivatives through 1,3-Dipolar Cycloadditions for applications in medicinal chemistry

4.1. Polysubstituted pyrazoles

It has been recently reported that that 3,5-substituted pyrazoles constitute a promising class of compounds for the design of kinase inhibitors targeting the ATP pocket of protein kinase.[41] Since we had developed a rapid, efficient and regio-controlled synthesis of polyfunctionalized pyrazoles, based on 1,3-DC of nitrile imines with functionalized acetylenes (see 2.1.), we sought that this methodology would be applicable for the preparation of such biologically active compounds.[42]

The followed approach towards the synthesis of substituted pyrazoles as kinase inhibitors involved a computer-assisted strategy in molecular design. Therefore, after extensive evaluation of a large number of derivatives obtainable through 1,3-DC, targets **67** and **68** were chosen as the most promising candidates, showing the lowest ranking binding energy towards the ATP binding site of a class of 8 selected target proteins kinases, involved in the HCC (Hepatocellualr Carcinoma) pathway. Compound **67** could form two hydrogen bonds into the binding site of the selected proteins, while **68** could form two or three ones.

Moreover, π-π hydrophobic and π -cation interactions, through the pyrazole rings and the aryl moieties, were present on both compounds docked with the selected kinases, increasing the binding affinity with the active site (Figure 11).

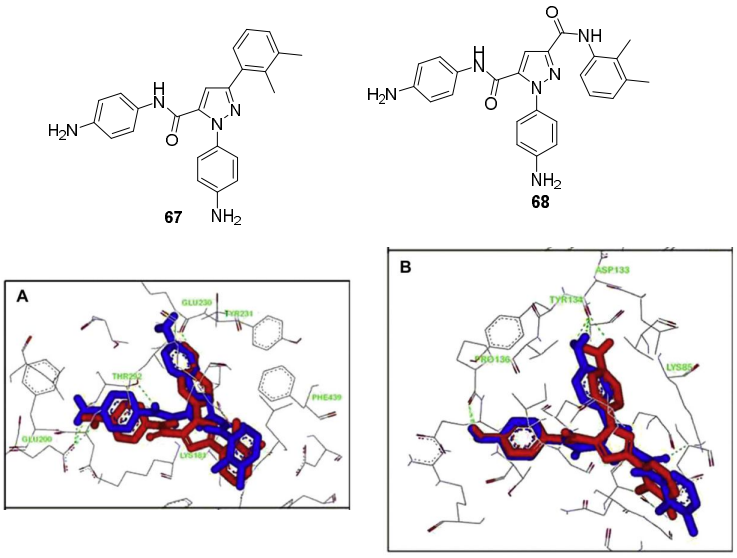


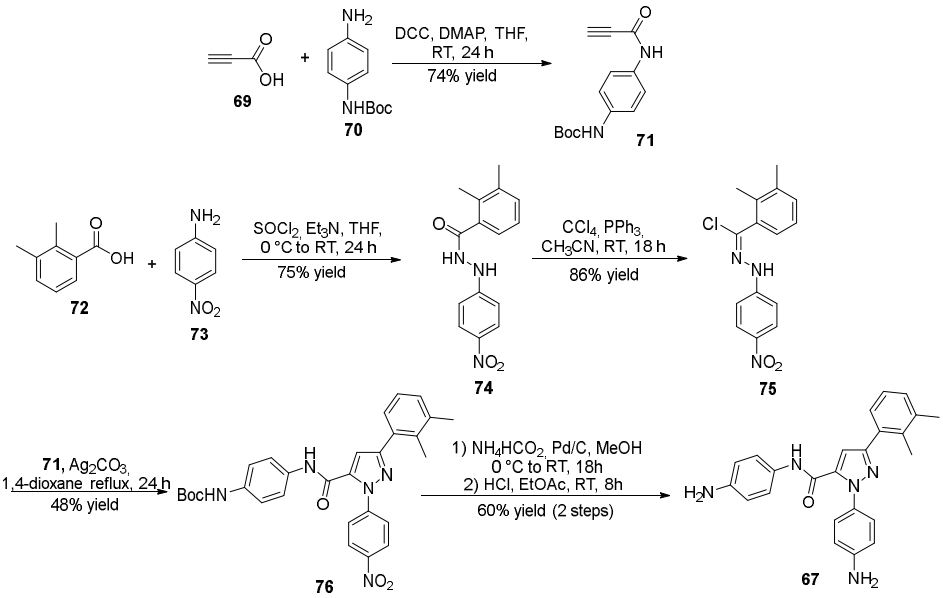
Figure 11. Targets **67** and **68**. **A**: Best docking poses of **67** (red) and **68** (blue) into the ATP binding site of AKT2 protein kinase. **B**: Best docking poses of **67** (red) and **68** (blue) into the ATP binding site of GSK-3β.

The synthesis of the selected compounds started with the preparation of the precursors for the 1,3-DC enabling the formation of the pyrazole ring. Substituted propiolamide **71** was prepared from propiolic acid **69** through coupling with *N*-*p*-Boc-phenylendiamine **70**. Hydrazonoyl chloride **75** was prepared from 2,3-dimethylbenzoic acid **72**, activated as acyl chloride and reacted with *p*-NO_2-aniline **73** to form hydrazide **74**, subsequently chlorinated under Appel conditions (CCl_4 and PPh_3).[43] Cycloaddition of **75** with **71**, treatment with Ag_2CO_3 in 1,4-dioxane at 80 °C for 24 h, as previously reported (see 2.1.), afforded pyrazole **76** as a single regioisomer in 48% yield. Nitro group reduction (hydrogenation with ammonium formate and Pd/C) and Boc group cleavage under standard acidic conditions furnished the desired target **67** in 60% combined yield (Scheme 10).

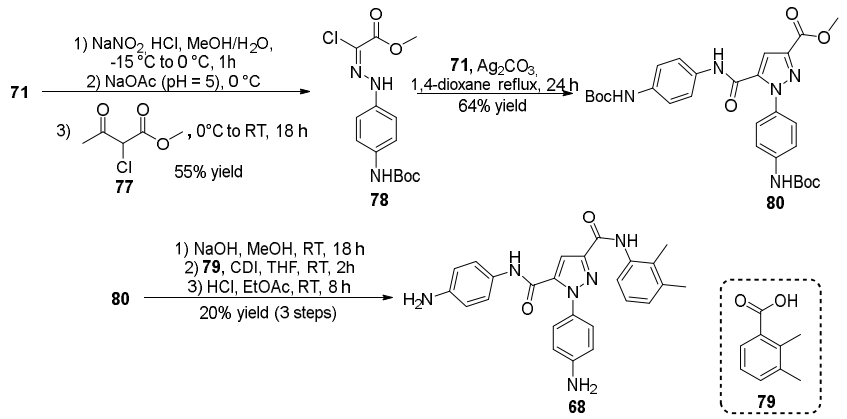
For the synthesis of **68**, hydrazonoyl chloride **78** was prepared by reaction of methyl 2-chloroacetoacetate **77** with **71**, previously converted in the corresponding diazonium salt with $NaNO_2$/HCl in methanol. Cycloaddition with **71**, under the previously mentioned conditions, afforded pyrazole **79** (along with a 20% of the corresponding 4-subsititued regioisomer, separated by column chromatography) in 64% isolated yield. Saponification of the ester moiety, reaction of the so-formed carboxylic acid with 2,3-dimethylaniline **80**, under the activation of CDI, and final Boc deprotection afforded compound **68** in 20% yield over three steps (Scheme 11).

First of all, the antiproliferative effect and cytotoxicity essay of compounds **67** and **68** was evaluated against SNU449 cells at a concentration of 50, 100 and 150 μM. Derivative **68** showed an IC_{50} of 150 μM at

48 h and of 50 μM at 72 h, while **67** had an IC_{50} of less than 100 μM at 48 h and less than 50 μM at 72 h, promising results for multi-kinase inhibitors.



Scheme 10. Synthesis of substituted propiolamide **71** and target **67**.



Scheme 11. Synthesis of target **68**.

To effectively prove that the target compounds acted as ATP-kinase inhibitors, as shown by the calculations of the molecular docking, the biological effect of **67** and **68** on the PI3K/AKT proteins phosphorylation pathway was evaluated. This molecular pathway is often deregulated in hepatocellular carcinoma and its involvement in HCC development and progression is well known. Thus, the phosphorylation levels of AKT downstream effectors in SNU449 cells treated with 100 μM **67** or **68** at 48 or

72 h, was evaluated by Western Blot analysis. This showed, in both cases, a consistent decrease in the phosphorylation of the targeted molecules, demonstrating that compounds **67** and **68** deregulate the PI3K/AKT pathway with a decrease of the AKT kinase activity.

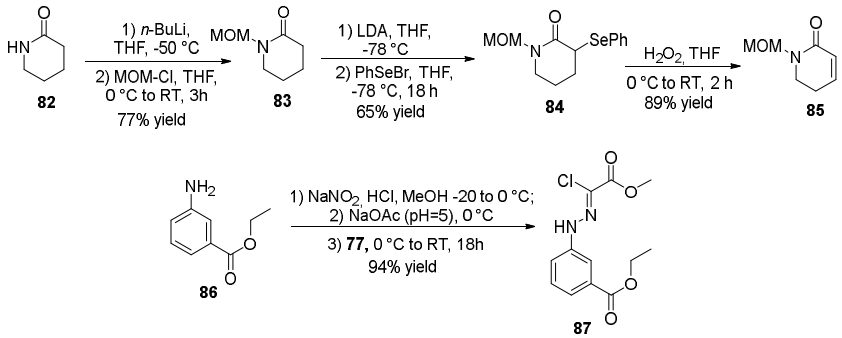
Moreover, it has been demonstrated, through cytofluorimetric analysis, that both the two molecules played an important role in cell cycle regulation of SNU449 cells, with a marked increase in the G1 phase and a decrease of the S phase.

The previous biological investigations indicate 3,5-disubstituted pyrazoles having in position 3 both aromatic and amidic functional groups as a new strategy to inhibit the PI3K/AKT pathway in hepatocellular carcinoma. This, taken together with the generality of the synthetic methodology presented, illustrates **67** and **68** as leading compounds for the construction of libraries of analogues, with the aim to improve the drug response to molecularly-targeted therapy agents.

4.2. Ring fused pyrazoles

Condensed pyrazole derivatives have often shown antiproliferative activities towards a wide number of carcinogenic cellular lines and are widely employed in drug discovery screening.[44] Lactams, whose importance arises primarily from the broad spectrum of applicability shown in modern industries,[45] have also assumed certain relevance as promising biologically active motives.[46] As part of our group's on-going effort directed towards the preparation of pyrazolic derivatives as cytotoxic agents, we planned the construction of a complex molecular target **81** with potential antitumor activity. The central scaffold, characterized by a lactam-fused pyrazole, would be constructed taking advantage of the 1,3-DC previously reported by the research group (see 3.2.). Moreover, in order to confer enhanced affinity for general biological environments and increased solubility to the target compound, we designed a "side chain" molecular fragment, containing a terminal piperidine, for a double late-stage functionalization.[47]

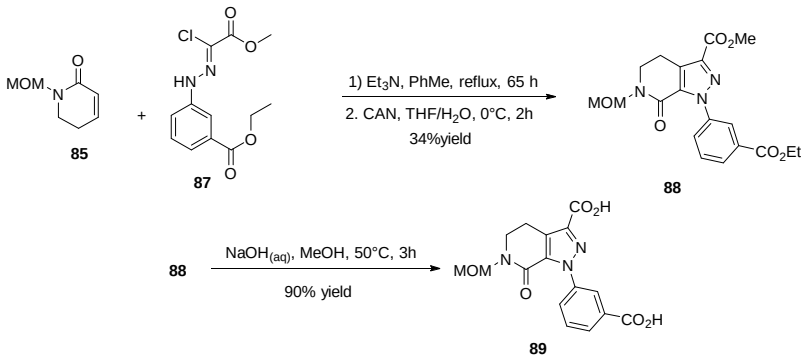
The synthesis started with the preparation of the dipolarophile (an α,β-unsaturated δ-lactam) and the precursor of the dipole (a hydrazonoyl chloride). As the first is concerned, reaction of commercially available 2-piperidone **82** with *n*-BuLi and MOM-Cl in THF, afforded protected lactam **83** in 77% yield. This was treated with LDA and PhSeBr in THF to afford (65% yield) α-selenylated product **84**, which underwent subsequent oxidation-retro cycloaddition upon reaction with H_2O_2. The desired α,β-unsaturated protected δ-lactam **85** was therefore obtained in 89% yield (Scheme 12).



Scheme 12. Preparation of dipole **85** and hydrazonoyl chloride **87**.

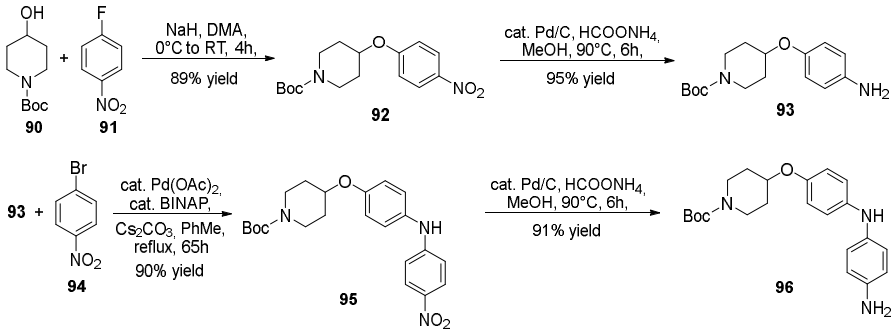
On the other hand, hydrazonoyl chloride **87** was synthesized with the same methodology described previously for **78** (see **4.1**), starting from ethyl 3-amino benzoate **86** and 2-chloroacetoacetate **77**, and obtained in 94% yield.

Cycloaddition-oxidation of **85** with **87** was carried out with slight modifications to the previously reported conditions (see **3.2**), obtaining pyrazole **88** (along with 15% of the other possible regioisomer, separated by column chromatography) in 34% isolated yield. This was reacted with NaOH in methanol to afford diacid **89** in 90% yield (Scheme 13).



Scheme 13. Synthesis of diacid **89** through 1,3-DC of **85** with **87** and saponification.

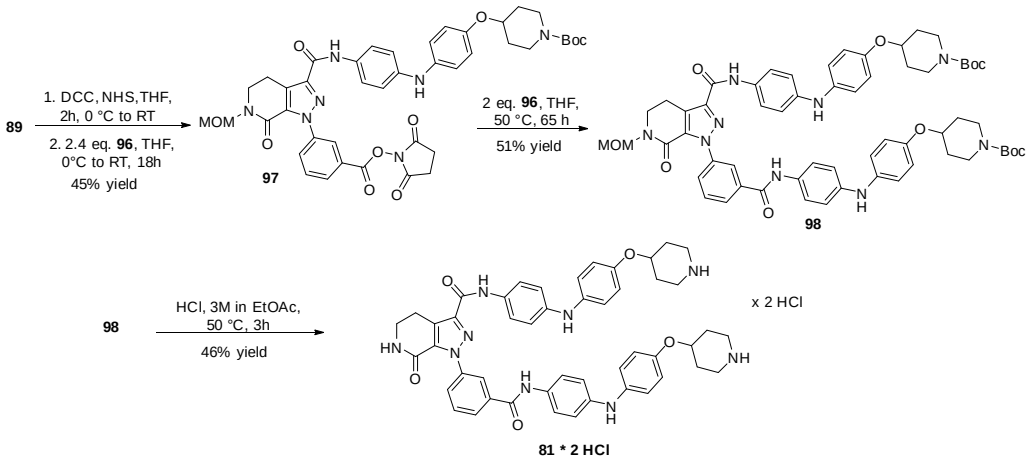
Preparation of the "side-chain" started with nucleophilic aromatic substitution of *N*-Boc-4-hydroxypiperidine **90** on *p*-F-nitrobenzene **91** to afford product **92**, which, upon subjection to standard hydrogenation conditions (ammonium formate and Pd/C in hot methanol) converted into amine **93**. Buchwald-Hartwig coupling of this substrate with *p*-Br-nitrobenzene **94** afforded product **95**, which was again hydrogenated under the same reaction conditions to render amine **96** in 69% yield over 4 steps (92% yield per step) (Scheme 14).



Scheme 14. Preparation of the "side chain", amine **96**.

Activation of diacid **89** with DCC and NHS in THF, followed by treatment with excess **96** afforded intermediate **97**. This proved to be surprisingly stable towards hydrolysis and column chromatography and

was therefore isolated in 45% yield. The possible isolation of this intermediate, along with the complete selectivity showed towards the activated carboxylic group of the pyrazole ring, opens the route for possible asymmetric functionalizations. In fact, **97** proved to be activated enough for a subsequent reaction with the application of only slightly harsher conditions: protected target **98** was therefore obtained in 51% yield upon treatment of **97** with excess **96** in THF at 50 °C for a prolonged time. Finally, simultaneous deprotection of MOM and Boc groups with HCl in hot EtOAc afforded final target **81** (triple hydrochloric salt) in 46% yield. A high purity sample was then obtained by preparative HPLC purification (Scheme 15).



Scheme 15. Obtainment of the final target **81**.

We next tested the activity of **81** on multiple cancer cell lines, such as HeLa (ovarian cancer), U87MG (glioblastoma) and HN13 (head and neck cancer). Compound **81** was dissolved in water and added to cell culture medium at different concentrations, from 0.01 to 100 µM. Cell viability was assessed after 24, 48 and 72 hours following treatment with **81** and, then, IC50 for each cell line was determined. The cytotoxic effect of **81** seemed to be concentration and time dependent for all the three cancer cell lines tested, suggesting that **81** could be an anti-proliferative compound. IC_{50} values at 72h ranged from about 16-33.5 µM, comparable with many commercially available anti-cancer drugs (Figure 13).

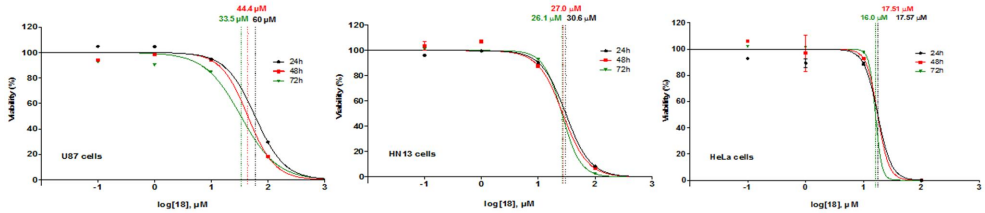


Figure 13.

5. Conclusions

In conclusion, we have highlighted our activity on the regio-controlled synthesis of pyrazoles *via* 1,3-dipolar cycloaddition of nitrile imines with several π-systems, both electron-rich and electron-deficient, such as triple bonds and α,β-unsaturated systems. The herein presented summary has shown that the regio-control of the reaction was possible by tuning substituents, selecting suitable substrates and using Lewis Acids. Molecular modelling helped in understanding the reaction pathway and the regio-chemistry.

This is an efficient example of the possibility to obtain diverse pyrazoles for applications in medicinal chemistry, as pointed out by the high in vitro cytotoxicity showed by the selected targets, on several cancer cell lines.

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