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THE REACTIVITY OF 4'-SUBSTITUTED SPIRO[ISOINDOLE-1,3'-PYRAZOLES] DERIVATIVES: SUBSTITUTION/ELIMINATION REACTIONS AND ACCESS TO BIARYL DERIVATIVES

Benedetta Maggio,[a] Gianfranco Fontana,[b] Maria Valeria Raimondi,[a] Demetrio Raffa,[a] Fabiana Plescia,[a] Angela Maria Ingarra,[a] and Giuseppe Daidone[a,*]

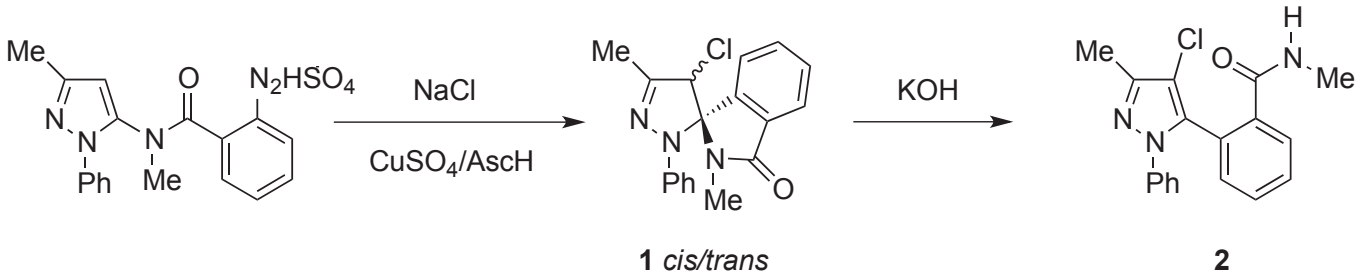
a. Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF); Medicinal Chemistry and Pharmaceutical Technologies Section, University of Palermo, via Archirafi 32, 90133 Palermo (Italy). E-mail: giuseppe.daidone@unipa.it
b. Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF); Chemistry Section, University of Palermo, viale delle Scienze, ed. 17, 90128 Palermo (Italy). E-mail: gianfranco.fontana@unipa.it

Abstract – This paper describes aspects of the chemistry of 4'-substituted spiro[isoindole-1,3'-pyrazoles]. These compounds underwent substitution and/or elimination reactions to afford some new spiro– as well as biaryl derivatives of potential pharmaceutical relevance. Mechanistic considerations are discussed as well.

INTRODUCTION

In the context of our research on the chemistry and pharmacology of pyrazole compounds,[1-15] we found that treatment of the diazonium salt of 2-amino-*N*-methyl-*N*-(3-methyl-1-phenylpyrazol-5-yl)benzamide with copper(II) sulfate, ascorbic acid and sodium chloride[9] leads to unusual frameworks of the type 4'-chloro-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one[8,9] **1** (Scheme 1). Furthermore, base treatment of the latter causes isomerization into 2-(4-chloro-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-methylbenzamide[8] (**2**, Scheme 1). Compounds of the type **1** exhibit both a spiropyrazoline and a spiroisoindole moiety. Spiropyrazoline systems are of interest as potential organic photoconductors[16] and exhibit promising bioactivity as antimicrobial[17] and

antiproliferative agents.[18] On the other hand, spiroisoindole subunits are found in neuropeptide Y receptor antagonists for the treatment of cardiovascular disorders, CNS disorders, and metabolic diseases.[19]



1 *cis/trans* **2**

Scheme 1. Intramolecular radical cyclization/chlorination of the diazonium compound followed by alkaline isomerization of **1** to give biaryl compound **2**

Furthermore, a diphenylpyrazole framework of the type **2** is the basic structural motif of the clinically relevant NSAID drug, Celecoxib®, a selective COX-2 inhibitor,[20] as well as of other agents endowed with diverse pharmacological properties, such as antiproliferative,[21] antimicrobial,[22] cannabinoid receptor 1 antagonistic,[23] HSP-90 inhibitory,[24] and anti-HIV-1[25] activities. Representative examples are shown in Figure 1.

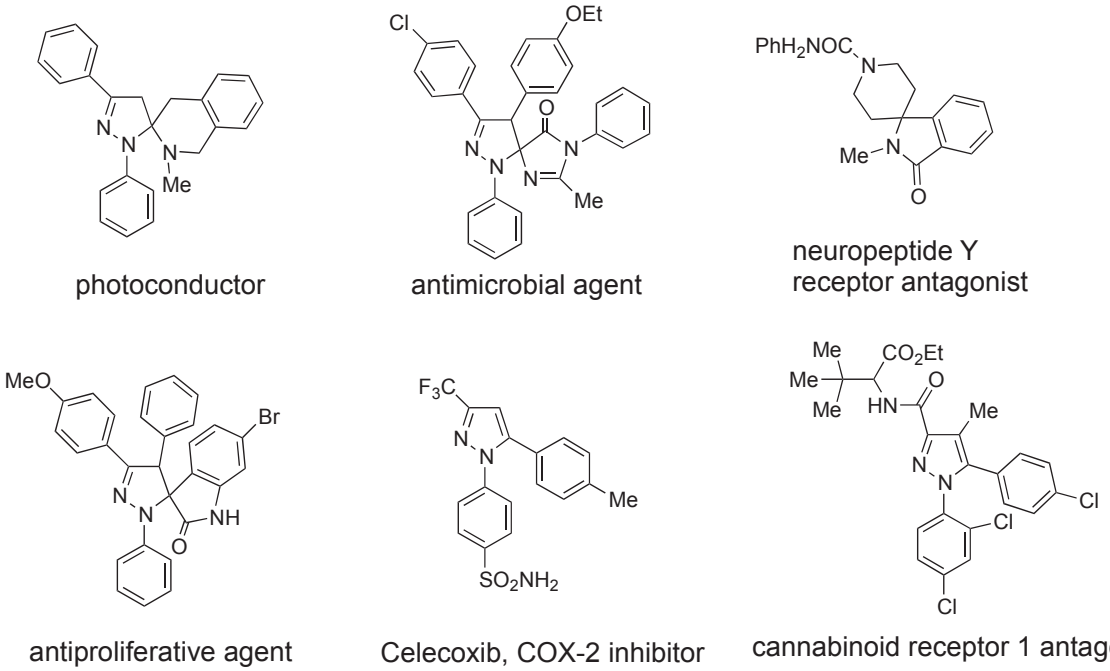


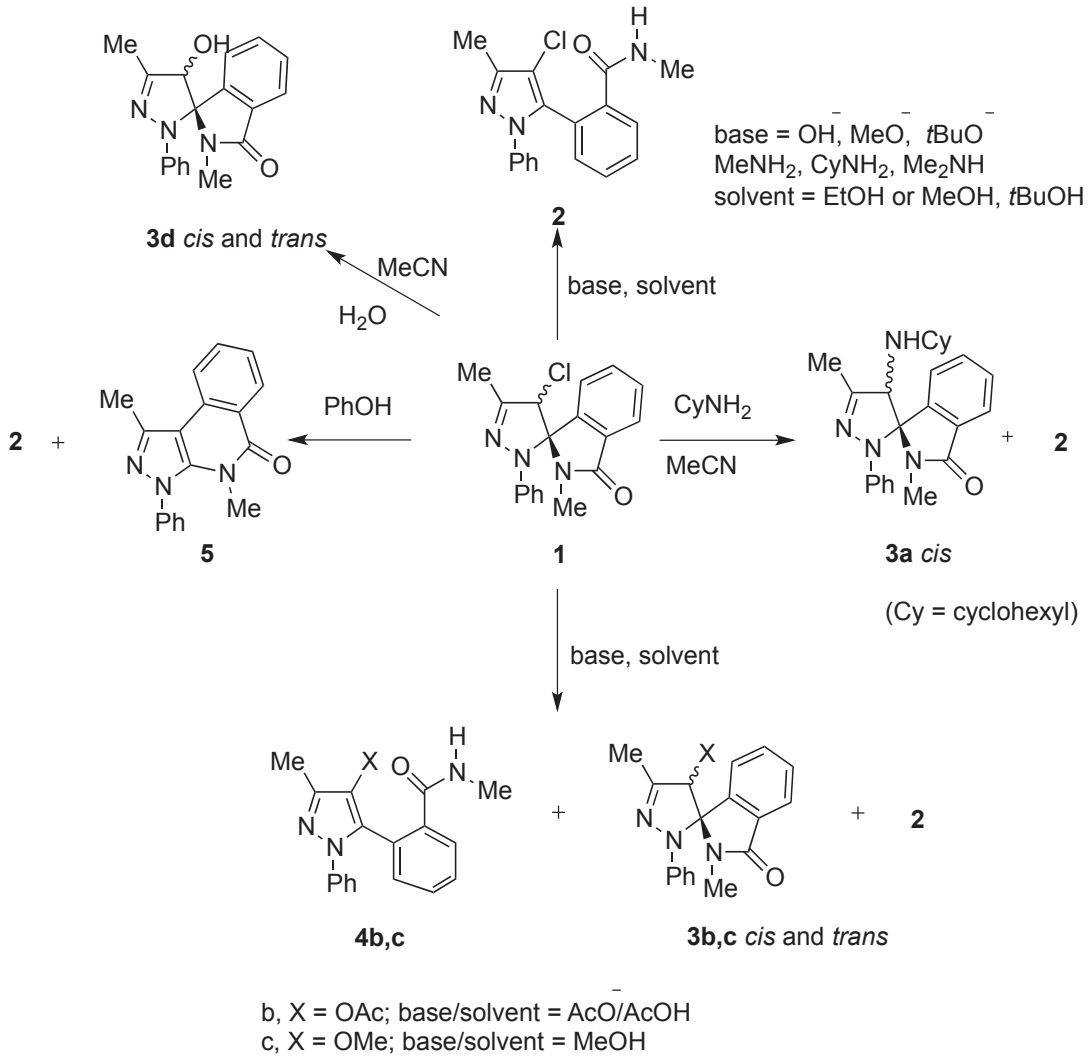
Figure 1. Examples of relevant compounds incorporating spiropyrazoline, spiroisoindole and diphenylpyrazole frameworks

In light of the above, we further explored the chemistry of compounds **1** in order to access a wider chemical diversity. Hence, we developed procedures to obtain 4'-substituted spirocompounds **3a-d**[8,9] and 4-substituted-1,5-diphenylpyrazoles **4a-d** (Scheme 2). We note that the preparation of the latter substances

by direct nucleophilic substitution of the C-4 chlorine atom in pyrazole **2** would be quite difficult under ordinary conditions.

RESULTS AND DISCUSSION

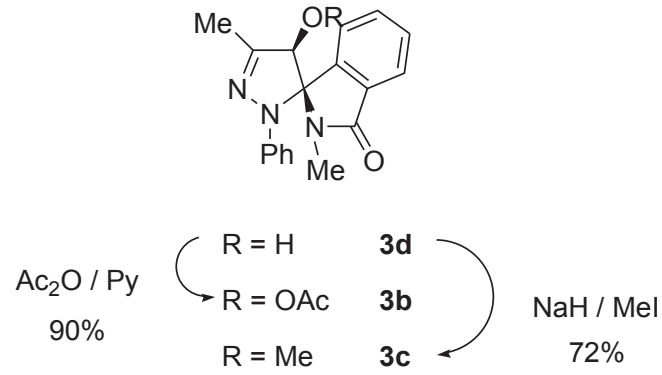
The mixture of epimers of **1** was prepared as previously reported.[9] Subsequent reaction with several nucleophiles (Scheme 2) under the conditions reported in Table 1 gave products of β-elimination (**2**) or substitution (**3a-d**); a single epimer of **3a** and both the epimers of **3b-d** were isolated. The latter are described as cis or trans isomers depending on whether group X is syn or anti to the spirolactam N atom.



Scheme 2. Action of bases/nucleophiles on the chloro-derivative **1** *cis/trans* to give 4'-substituted spiro derivatives **3a-d** and 4-substituted-1,5-diphenylpyrazole derivatives **4b,c**

Reactions leading to **3b,c** also produced variable amounts of **2** and **4b,c**. Dissolution of **1** in molten phenol resulted in formation of mostly **2**, accompanied by the interesting by-product **5**.[26,27] In each case, both epimers of **1** were completely consumed. The relative configuration of the stereoisomers of **3b,c** was

assigned by chemical correlation (Scheme 3) with the epimers of **3d**, the stereochemistry of which is known.[9] Hence, a stereochemically pure sample of (3'*R*,4'*S*)-4'-hydroxy-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (*cis*-**3d**) was methylated (NaH / MeI, THF, 0 °C, 2 to give *cis*-**3c**, or acetylated (Ac_2O / pyridine, rt, 1 h) to give *cis*-**3b**. Compounds *cis*-**3b**,**c** thus obtained were identical in all the respect to the major product (less polar by silica gel TLC) obtained in entries 8 9.



Scheme 3. Chemical correlation of the epimers of the spiro derivatives **3b,c** with the known compound *cis*-**3d**

The composition of the product mixtures appears to be a function of the degree of Brönsted basicity of the reaction medium: the more basic the medium, the greater the extent of elimination (Table 1).

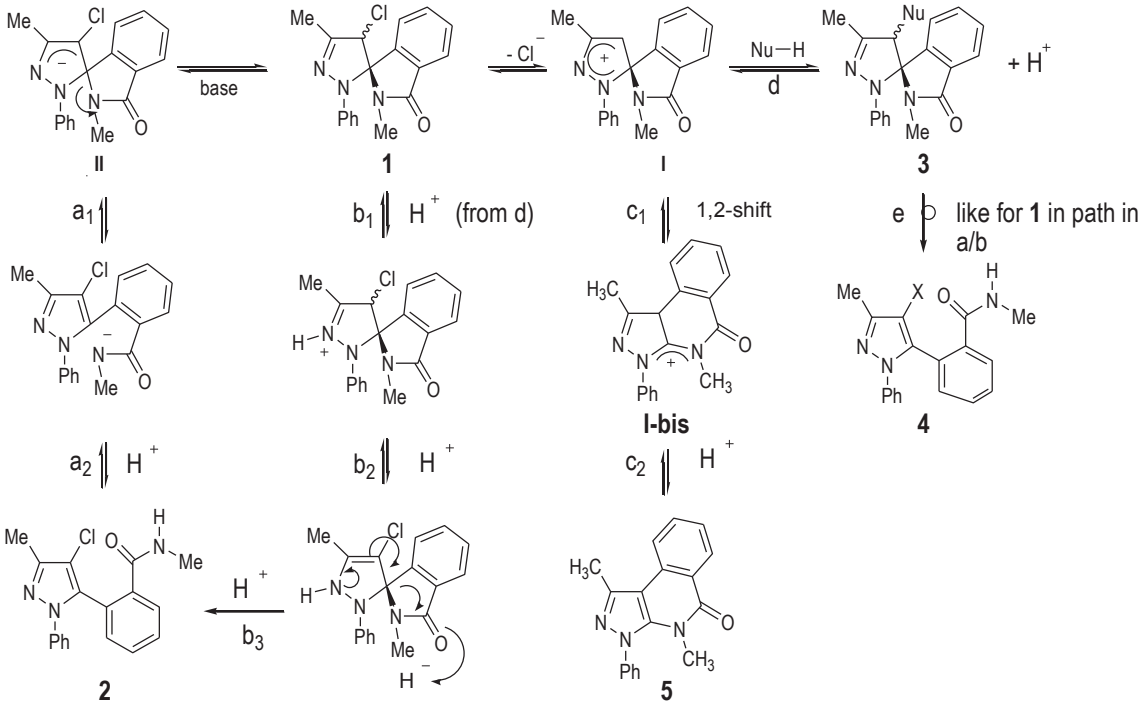
Table 1. Products preparative yields and experimental conditions in the treatments of compound 1[a]

Entry	Solvent	Reagents	T (t)	% **2**	% **3**	% **4**
1	EtOH	OH^-	rt (5')	82	0	0
2	MeOH	MeO^-	rt (5')	80	0	0
3	*t*BuOH	*t*BuONa	rt (5')	81	0	0
4	EtOH	Me_2NH	rt (24 h)	85	0	0
5	EtOH	$MeNH_2$	rt (24 h)	80	0	0
6	EtOH	$CyNH_2$	rt (24 h)	82	0	0
7	MeCN	$CyNH_2$	reflux (11 h)	11	*cis*-**3a** (37)	0
8	AcOH	AcO^-	reflux (8 h)	3	**3b** (21 *cis*, 15 *trans*)	**4b** (19)
9	MeOH	MeOH	reflux (8 h)	26	**3c** (29 *cis*, 18 *trans*)	**4c** (16)
10	MeCN/H_2O	H_2O	reflux (30')	0	**3d** (50 *cis*, 22 *trans*)	0
11[b]	PhOH	PhOH	100 °C (3 h)	40	0	0

a. Initial epimeric ratio: 2.2/1 *cis/trans*. b. Compound **5** was also obtained, 9%.

Thus, bases such as hydroxide, alcoxides (methoxide, *t*-butoxide), amines (methylamine, cyclohexylamine and dimethylamine) in ethanol, reacted with **1** to afford only the elimination product **2** (entries 1-6). This is because the specific base catalyzed removal of the H-4 proton, that in the cases involving amines in ethanol (entries 4-6) is due to the in situ generated ethoxide anion, is faster than the substitution. In contrast, cyclohexylamine in MeCN (entry 7) can act both as a nucleophile in a substitution reaction leading to product **3a** and as a base affording the elimination compound **2**. The reduced basicity of acetate anion relative to alkoxydes, or ethanolic amines, translated into a largely dominant substitution reaction (entry 8) upon exposure of **1** to NaOAc in refluxing acetic acid. However, a significant amount of compound **4b** originated from the initially formed substitution products **3b** (*cis* and *trans*) by a subsequent elimination step. The potential role of acetic acid in the latter transformation will be addressed shortly (*vide infra*). Comparable quantities of all possible reaction products were formed when a methanolic solution of **1** was heated at reflux (entry 9); that is, compounds **3c** (*cis* and *trans*), **2**, and **4c**, the latter originating from **3c**. A solvent change from methanol to aqueous MeCN (entry 10), resulted in formation of alcohols **3d** (*cis* and *trans*) only, without even a trace of the elimination products. Finally, dissolution of **1** in molten phenol (entry 11) resulted in the formation of mostly **2**, accompanied by a small quantity of the known lactam **5**. The electronic properties of the pyrazoline ring in **1** enable it to stabilize both carbocation **I** and carbanion **II** (Scheme 4). Under basic conditions the formation of **II** dominates, promoting an E1cb elimination reaction (path a), in which the conjugate base of the carbamoyl group functions as the leaving group (step a_1). Conversely, polar, less basic media (H_2O/MeCN, MeCN, MeOH, PhOH, AcOH) promote dissociation of **1** leading to cation **I**, which then furnishes SN_1-type products **3a-d** upon reaction with the appropriate nucleophile (path d). In the case of methanol or water, such an event also results in formation of protonated solvent molecules (H_3O^+, $MeOH_2^+$), which then may promote acid-catalyzed reactions. For instance, protonation of the carbonyl group of **1** or **3** may trigger eliminative aromatization with formation of **2** (path b) or **4** (path e). This is probably the reason why reaction of **1** with NaOAc/AcOH afforded elimination products **2** and **4b** in addition to **3b**, although, of course, one cannot exclude the occurrence of elimination promoted by acetate anion via path a. Furthermore, the formation of elimination product **2** in MeOH, not observed in H_2O, (Table 1) is attributable to the greater acidity of $MeOH_2^+$ with respect to H_3O^+ (ΔpKa ca. - 2).[28] In contrast, the nucleophilicities of water and methanol are substantially comparable.[29] Thus, $MeOH_2^+$ is a more effective acid catalyst in steps b1 and e. Support for this conclusion is provided by the observation that compound *cis*-**3c** was partly converted into **4c** upon refluxing for 8 h in dilute methanolic HCl solution. With respect to the reaction of **1** in molten phenol, product **2** probably results upon acid catalyzed elimination (steps b1, b2). A rationale for the formation of **5** envisions that the low

nucleophilicity of phenol translates into a longer lifetime for cationic intermediate **I**, which may now rearrange by a 1,2-aryl ring shift in preference to nucleophilic trapping (path c).



Scheme 4. Mechanistic proposal for the formation of the various products

The foregoing discussion is relevant to stereochemical aspects of the substitution process, which in all cases afforded the *cis* isomer of **3b-d** as the dominant product. This stereoselectivity can be explained by invoking an attack of the incoming nucleophile from the less sterically hindered face of cation **I** syn to the lactam nitrogen (Figure 2). We do not have any direct evidence for the configuration of compound **3a**. However, considering that this substance was obtained as a single stereoisomer, it seems reasonable that the steric demand of the nucleophile (cyclohexylamine) should strongly favor the formation of *cis*-**3a**.

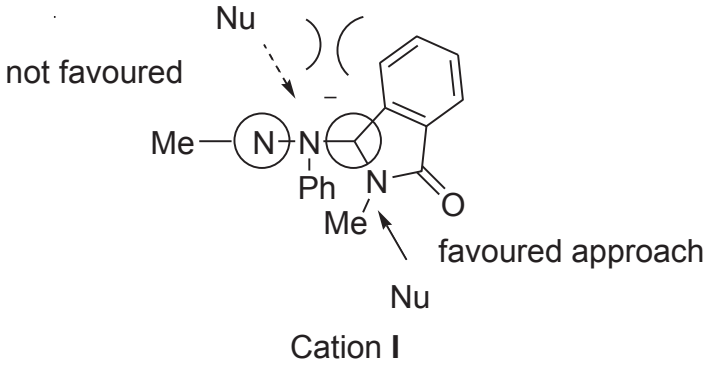
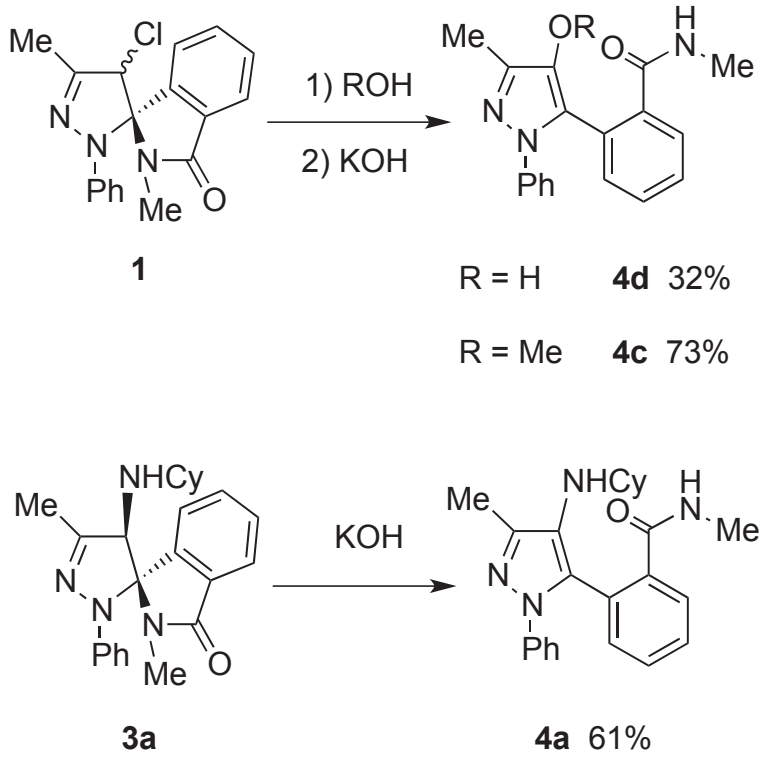


Figure 2. Differential approach of the incoming nucleophile toward cation **I**

It will be recalled that compounds **3b,c** were accompanied by secondary products **4b,c**, whereas no such by-products were obtained in the case of **3a,d** (see Scheme 2). Considering the pharmaceutical interest of derivatives of the type **4**, experiments aiming to produce **4a,d** and to improve the yields of **4b,c** were carried out. The mechanistic outline of Scheme 4 suggests that treatment with strong base should induce conversion of compounds **3** into elimination products **4** (cf. step e). Indeed, substance **3a** was quantitatively converted into **4a** upon reaction with KOH in ethanol (Scheme 5). An attempt to achieve the same transformation with the acetate derivatives **3b** resulted in formation of compounds **3d** instead of **4b**. This is due to a fast hydrolysis of the acetate ester under basic conditions. Furthermore, a two-step/one-pot procedure enabled the direct advancement of the mixture of epimers of **1** to compounds **4c,d**, via sequential treatment with a suitable nucleophile, followed by KOH in ethanol (Scheme 5). Under these conditions, the primary products **3c,d** underwent elimination *in situ*.



Scheme 5. Preparation of biaryl derivatives by direct one-pot substitution/isomerization of compound **1** to give **4c,d** and alkaline isomerization of **3a** to give **4**

In conclusion, the results obtained in this study reveal some general aspects of the chemistry of the 4'-substituted-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one derivatives, giving access to new compounds of this type and to 4-substituted-1,5-diarylpyrazoles of potential pharmaceutical interest.

EXPERIMENTAL

General experimental procedures

Reaction progress was monitored by TLC on silica gel plates (Merck 60, F_{254}, 0.2 mm). Organic solutions were dried over Na_2SO_4. Evaporation refers to the removal of solvent on a rotary evapora-tor under reduced pressure. All melting points were determined on a Büchi 530 capillary melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin Elmer Spectrum RXI FT-IR System spectrophotometer as solid in KBr disc or Nujol. ^{1}H-NMR and ^{13}C-NMR spectra of all compounds were obtained in $CDCl_3$ or DMSO-*d*$_6$ solutions on a Bruker AC 250 F or a Bruker Avance II 400 spectrometers and in all cases tetramethylsilane was used as an internal standard; chemical shifts are expressed in δ values (ppm). Mass spectra at 70 eV were obtained using an 3400-CX Varian gas chromatograph fitted with a Saturn 3 mass spectrometer. Merck silica gel (Kiesegel 60/230-400 mesh) was used for flash chromatography (FC) columns. Yields refer to products after one crystallization. Microanalysis data (C,H,N) were obtained by an Elemental Vario EI. III apparatus and were within ± 0.4% of the theoretical values. The name of the compounds were obtained using the ChemDraw Ultra 12.0 software. The epimers 4'-chloro-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (**1**) as a mixture were prepared accordingly to a previously published procedure.[9]

Action of KOH/EtOH on the 4'-chloro-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-ones (1 *cis/trans*).

100 mg (0.31 mmol) of **1***cis*/*trans* were solubilized in 5 mL of a 1 M solution of KOH in 95% EtOH. After 5 min of stirring the solution was neutralized with acidic EtOH and the solvent was evaporated. The residue was extract with $CHCl_3$ (5 mL), and the solution was evaporated affording a solid which was recrystallized from 95% EtOH giving a pure product which was identical in all respect to **2**[8] (82%).

Action of MeONa/MeOH or *t*BuONa/*t*BuOH on the 4'-chloro-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-ones (1 *cis/trans*).

115 mg (5 mmol) of Na were reacted in 5 mL of MeOH or *t*BuOH under Ar; then 100 mg (0.31 mmol) of **1** *cis*/*trans* were added in one portion. After 5 min of stirring the solution was neutralized with acidic MeOH (HCl) and the solvent was evaporated. The residue was extract with $CHCl_3$ (5 mL), and the solution was evaporated affording a solid which was recrystallized from 95% EtOH giving a pure product which was identical in all respect to **2**[8] (80% and 81% respectively).

Action of $MeNH_2$, Me_2NH and cycloexylamine on the epimers 1 *cis*/*trans*, in EtOH.

100 mg (0.306 mmol) of **1** *cis*/*trans* were dissolved in 3 mL of a 1 M EtOH solution of $MeNH_2$ or Me_2NH or cyclohexylamine. The solution was stirred at rt for 24 h. The volatiles were removed by evaporation under vacuum and the solid residue was recrystallized from EtOH giving compound **2** (80% for $MeNH_2$, 85% for Me_2NH and 82% for cycloexylamine).

Action of cyclohexylamine on the epimers 1 *cis*/*trans*, in MeCN.

To a solution of 500 mg (1.53 mmol) of **1** *cis*/*trans* in MeCN (25 mL), 493 μL (427 mg, 4.59 mmol) of cyclohexylamine were added in one portion. The resulting solution was refluxed for 11 h and the volatiles were removed by evaporation under vacuum. The residue was purified by FC (EtOAc/light petroleum, bp 40-70 °C 3:7 v/v as eluent) to give practically pure *cis*-**3a** (37%) and crude **2** which was recrystallized from EtOH (11%).

(3'*S*,4'*R*)-*rel*-4'-Cyclohexylamino-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (*cis*-3a): pale yellow solid; mp 116-119 °C (Et_2O); IR (KBr, cm^{-1}) 3332, 1685; ^1H NMR (250 MHz, $CDCl_3$) δ 0.64 – 2.00 (m, 12H), 2.15 (s, 3H), 3.00 (s, 3H), 4.34 (s, 1H), 6.69-7.88 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.3, 25.8, 69.5, 73.0, 115.5, 121.6, 123.8, 1 23.9, 128.8, 129.6, 131.5, 132.6, 14 3.9, 147.0, 152.5, 167.8. MS *m/z* 388 (M^+). Anal. Calcd for $C_{24}H_{28}N_4O$: C, 74.20; H, 7.26; N, 14.42). Found: C, 74.31; H, 7.38; N, 14.90.

Action of acetate on the epimers 1 *cis*/*trans*.

To a solution of 208 mg (2.53 mmol) of NaOAc, solubilized in 15 mL of AcOH, 500 mg (1.53 mmol) of epimers **1** *cis*/*trans* were added in one portion. The resulting solution was refluxed for 8 h. Afterward, the solid residue was removed by filtration and the filtrate was distilled under reduced pressure. The residue was extracted with Et_2O (3x40 mL) and the solution obtained was evaporated under vacuum affording a solid which was crystallized from EtOH to give compounds **3b** as an epimeric mixture. The mixture was chromatographed (FC) (Et_2O/ light petroleum, bp 40-70 °C, 7:3 v/v) to give the pure stereoisomer *cis*-**3b** (17%) and crude *trans*-**3b** which was chromatographed with the above eluent to give pure *trans*-**3b** (11%). The mother liquors obtained from the crystallization were evaporated under vacuum and the residue was chromatographed (FC) (Et_2O as eluent) affording further small amounts of *cis*-**3b** (4%), *trans*-**3b** (4%), **2** (3%) and **4b** (19%).

(3'*S*,4'*R*)-*rel*-4'-Acetoxy-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (*cis*-3b): overall yield 21%, colorless solid, mp 163-165 °C (Et_2O); IR (KBr, cm^{-1}) 1749 and 1714; ^1H NMR (250 MHz, $CDCl_3$) δ 2.03 (s, 3H) 2.15 (s, 3H), 2.75 (s, 3H), 6.27-7.67 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.1, 20.1, 26.8, 29.7, 82.0, 115.3, 121.6, 123.9, 124.6, 128.9, 129.9, 131.4, 134.9, 142.8, 145.4, 146.5, 167.3, 168.8. MS *m/z* 349 (M^+). Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03). Found: C, 69.03; H, 5.55; N, 12.43.

(3'*S*,4'*S*)-rel-4'-Acetoxy-2,5'-dimethyl-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (*trans*-3b): colorless solid, mp 178-180 °C (Et_2O) IR (KBr, cm^{-1}) 1756, 1708; ^1H NMR (250 MHz, $CDCl_3$) δ 1.81 (s, 3H) 2.19 (s, 3H), 2.93 (s, 3H), 6.01 (s, 1H), 6.66-7.75 (m, 9H); ^{13}C NMR (75 MHz, CDCl3) δ 13.6, 20.2, 24.9, 29.7, 81.6, 115.5, 121.7, 124.0, 124.6, 128.9, 130.1, 131.4, 132.7, 139.4, 142.5,

145.8, 166.7, 168.8. MS *m/z* 349 (M^+). Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03). Found: C, 68.95; H, 5.61; N, 12.35.

2-(4-Acetoxy-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-methylbenzamide (4b): colorless solid, mp 168-170 °C (EtOH); IR (KBr, cm^{-1}) 3372, 1758, 1643; ^1H NMR (250 MHz, $CDCl_3$) δ 2.17 (s, 3H) 2.30 (s, 3H), 2.63 (s, 3H), 6.47 (s br., 1H), 7.02-7.64 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.5, 20.3, 26.8, 123.9 (2C), 125.3, 127.7, 129.1 (2C), 129.9, 130.1 (2C), 130.5, 132.1, 133.2, 136.7, 138.6, 141.2, 167.4, 170.4. MS *m/z* 349 (M^+). Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03). Found: C, 69.01; H, 5.59; N, 12.23.

Action of methanol on the epimers 1 *cis*/*trans*.

500 mg (1.53 mmol) of epimers **1** *cis*/*trans* were refluxed in dry MeOH (25 ml) for 10 h. The solvent was evaporated and the residue was purified by FC (EtOAc/light petroleum, bp 40-70 °C, 3:7 to 6:4 v/v, and then EtOAc as eluents) to give pure stereoisomers *cis*-**3c** (29%), *trans*-**3c** (18%), **4c** (17%) and **2** (26%).

(3'*S*,4'*R*)-*rel*-2,5'-Dimethyl-4'-methoxy-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (*cis*-3c): colorless solid, mp 176-178 °C (benzene/light petroleum, bp 40-70 °C); IR (KBr, cm^{-1}) 1695; ^1H NMR (250 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.57 (s, 3H), 3.03 (s, 3H), 5.20 (s, 1H), 6.53-8,86 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.0, 26.9, 60.3, 86.9, 92.5, 115.3, 121.5, 123.1, 124.2, 128.9, 129.8, 131.8, 132.6, 143.3, 146.9, 149.1, 167.3. MS *m/z* 321 (M^+). Anal. Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08). Found: C, 69.96; H, 6.05; N, 13.38.

(3'*S*,4'*S*)-*rel*-2,5'-Dimethyl-4'-methoxy-2'-phenyl-2',4'-dihydrospiro[isoindole-1,3'-pyrazol]-3(2*H*)-one (*trans*-3c): colorless solid, mp 153-154 °C (EtOAc/light petroleum, bp 40-70 °C); IR (KBr, cm^{-1}) 1686; ^1H NMR (250 MHz, $CDCl_3$) δ 2.15 (s, 3H), 2.83 (s, 3H), 2,86 (s, 3H), 4.96 (s, 1H), 6.56-7.79 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6, 24.6, 59.5, 88.4, 91.8, 115.5, 121.3, 123.5, 125.2, 128.8, 129.7, 131.8, 132.3, 139.6, 142.9, 144.6, 166.9. MS *m/z* 321 (M^+). Anal. Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08). Found: C, 71.11; H, 6.00; N, 13.25.

2-(4-Methoxy-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-methylbenzamide (4c): colorless solid, mp 176 °C (EtOH); IR (KBr, cm^{-1}) 3294, 1639; ^1H NMR (250 MHz, $CDCl_3$) δ 2.24 (s, 3H), 2.58 (d, J = 5.48 Hz, 3H), 3.52 (s, 3H), 7.10-7.50 (m, 9H), 8.08 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.1, 26.8, 62.1, 123.8, 126.6, 127.1, 128.5, 128.8, 129.1, 130.2, 130.9, 131.6, 137.3, 139.8, 141.0, 142.4, 168.6. MS *m/z* 321 (M^+). Anal. Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08). Found: C, 70.89; H, 6.05; N, 13.30.

Action of phenol on the epimers 1 *cis*/*trans*.

500 mg (1.53 mmol) of epimers **1** *cis*/*trans* were mixed with 8.63 gr (91.8 mmol) of phenol. The mixture was heated to 100 °C and kept at this temperature for 3 h. Afterward the molten mixture was cooled to rt and the solid residue was purified by FC (EtOAc/light petroleum, bp 40-70 °C 4:6 as eluent) to give

compound **2** (40%) and another compound (9%) which was identical in all respect to an authentic specimen of 1,4-dimethyl-3-phenylpyrazolo[3,4-*c*]isoquinolin-5-one (**5**) (TLC, ^{1}H-NMR, mixed mp).[26,27]

Action of water on the epimers 1 *cis*/*trans*.

150 mg (0.488 mmol) of epimers **1** *cis*/*trans* were refluxed in a water MeCN mixture (1:1; 8 mL) for 1 h. The solvent was evaporated and the residue was purified by FC (EtOAc/cyclohexane, 7:3 as eluents) to give pure compounds that were identical in all respect (TLC, ^{1}H-NMR, mixed mp) to authentic specimens of the stereoisomers *cis*-**3d** (the less polar, 70 mg, 50%) and *trans*-**3d** (the more polar, 32 mg, 22%) respectively.[9]

Preparation of 2-(4-cyclohexylamino-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-methylbenzamide (4a) from 3a.

A solution of **3a** (80 mg, 0.206 mmol) in 0.1 N alcoholic KOH (2 mL) was refluxed for 10 mins. To the solution was added water (3 mL) and the mixture was then extracted with Et_2O (3 x 5 mL). The organic layers were evaporated to obtain a crude oily residue (80 mg) which was crystallized from EtOAc/petroleum ether bp 40-60 °C to give 48.6 mg of compound **4a** (61%). Colorless solid, mp 129-130 °C, IR (Nujol, cm^{-1}) 3360, 1625; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 – 1.08 (m, 5H), 1.42 – 1.66 (m, 3H), 1.83 (m, 2H), 2.30 (s, 3H), 2.52 (m, 1H), 2,69 (s, 3H), 7.17-7.27 (m, 5H), 7.45 (m, 2H), 7.55 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.7, 26.8, 27.1, 27.2, 35.1, 58.8, 126.0, 128.3, 129.6, 129.8, 130.1, 130.2, 130.4, 131.6, 133.5, 135.6, 139.0, 141.4, 145.1, 171.6. MS *m/z* 388 (M^+). Anal. Calcd for $C_{24}H_{28}N_4O$: C, 74.20; H, 7.26; N, 14.42). Found: C, 74.31; H, 7.38; N, 14.60.

Direct preparation of 2-(4-methoxy-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-methylbenzamide (4c) from 1 *cis*/*trans*.

A solution of epimers **1** *cis*/*trans* (100 mg, 0.307 mmol) in 5 mL of absolute MeOH was refluxed for 10 h and then solid KOH (0.280 gr) was added. The solution obtained was refluxed for other 3 h and then evaporated under vacuum to give a residue which was washed with water (2 mL) and filtered off to obtain a mixture containing compounds **2** and **4c**. The mixture was cromatographed by FC (EtOAc/petroleum ether bp 40-60 °C 3:2 as eluent) to give 72 mg (73%) of pure **4c**.

Direct preparation of 2-(4-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-*N*-methylbenzamide (4d) from 1 *cis*/*trans*.

A solution of epimers **1** *cis*/*trans* (150 mg, 0.488 mmol) in MeCN/water (1:1; 8mL) was refluxed for 30 minutes and then evaporated under vacuum. To the residue was added a 1 N alcoholic KOH solution (3 mL) and the mixture was refluxed for 3 h and then evaporated under vacuum. The residue obtained was treated with water (4 mL), the pH was adjusted to 5-6 affording a suspension which was filtered out. The white solid obtained was crystallized from EtOH to give 45 mg (32%) of pure compound **4d**. Colorless

solid, mp 210-212 °C (EtOH); IR (Nujol, cm^{-1}) 3475, 3198, 1630; ^1H NMR (400 MHz, CD_3OD) δ 2.28 (s, 3H), 2.71 (s, 3H), 3.31 (m, 1H), 3.35 (br s, 1H), 7.13-7.28 (m, 6H), 7.41 (m, 1H), 7.48 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 10.6, 27.2, 125.7, 128.1, 129.3, 129.5, 130.0, 130.2, 130.3, 131.6, 133.6, 138.6, 140.2, 140.9, 141.6, 172.6. MS *m/z* 308 $(M+1)^+$. Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67). Found: C, 70.25; H, 5.61; N, 13.71.

Preparation of *cis*-3b from *cis*-3d.

50 mg (0.325 mmol) of pure *cis*-**3d** were solubilized in 3 mL of Ac_2O and 15 μL of dry pyridine were added. After 1 h the volatile material was removed by rotavapor and the residue was purified by FC (Et_2O as eluent) giving 51 mg (90%) of pure *cis*-**3b**, with mp (163-165 °C), rf and ^1H-NMR identical to those of the less polar stereoisomer (silic agel TLC) obtained from epimers **1** *cis*/*trans* and NaOAc.

Preparation of *cis*-3c from *cis*-3d.

100 mg (0.325 mmol) of pure *cis*-**3d** were solubilized in 10 mL of a 1:1 v/v mixture of dry THF and methyl iodide. Then 0.423 mmol (17 mg of a 60% w/w dispersion) of NaH were slowly added at 0 °C. The mixture was stirred under Ar for 2 h. Then the reaction mixture was slowly poured into 10 mL of saturated iced NH_4Cl solution and extracted with EtOAc (4 X 5 mL). The organic phase was washed with brine and distilled by rotavapor. The solid residue was recrystallized by benzene/light petroleum, bp 40-70 °C to give 75 mg (72%) of pure *cis*-**3c**, with mp (176-178 °C), rf and ^1H NMR identical to those of the less polar stereoisomer (silica gel TLC) obtained from the epimers **1** *cis*/*trans* and MeOH.

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REFERENCES

1. B. Maggio, M. V. Raimondi, D. Raffa, F. Plescia, S. Cascioferro, G. Cancemi, M. Tolomeo, S. Grimaudo, and G. Daidone, *Eur. J. Med. Chem.*, 2015, **96**, 98.
2. B. Maggio, D. Raffa, M. V. Raimondi, M. G. Cusimano, F. Plescia, S. Cascioferro, G. Cancemi, M. Lauricella, D. Carlisi, and G. Daidone, *Eur. J. Med. Chem.*, 2014, **72**, 1.
3. M. V. Raimondi, B. Maggio, D. Raffa, F. Plescia, S. Cascioferro, G. Cancemi, D. Schillaci, M. G. Cusimano, M. Vitale, and G. Daidone, *Eur. J. Med. Chem.*, 2012, **58,** 64.
4. D. Raffa, O. Migliara, B. Maggio, F. Plescia, S. Cascioferro, M. G. Cusimano, G. Tringali, C.

Cannizzaro, and F. Plescia, *Arch. Pharm. Chem. Life Sci.*, 2010, **343**, 631.

5. D. Raffa, B. Maggio, S. Cascioferro, M. V. Raimondi, G. Daidone, S. Plescia, D. Schillaci, M. G. Cusimano, L. Titone, C. Colomba, and M. Tolomeo, *Arch. Pharm. Chem. Life Sci.*, 2009, **342**, 265.
6. B. Maggio, D. Raffa, M. V. Raimondi, S. Cascioferro, F. Plescia, M. Tolomeo, E. Barbusca, G. Cannizzaro, S. Mancuso, and G. Daidone, *Eur. J. Med. Chem.*, 2008, **43**, 2386.
7. D. Schillaci, B. Maggio, D. Raffa, G. Daidone, S. Cascioferro, M. G. Cusimano, and M. V. Raimondi, *Chemotherapy*, 2008, **54**, 456.
8. G. Daidone, S. Plescia, B. Maggio, V. Sprio, F. Benetollo and G. Bombieri, *J. Chem. Soc., Perkin Trans. 1,* 1993, 285.
9. G. Daidone, B. Maggio, D. Raffa. S. Plescia, F. Benetollo, and G. Bombieri, *J. Chem. Soc., Perkin Trans 1,* 1998, 2891.
10. B. Maggio, G. Daidone, D. Raffa, S. Plescia, G. Bombieri, and F. Meneghetti, *Helv. Chim. Acta*, 2005, **88**, 2272.
11. B. Maggio, D. Raffa, M. V. Raimondi, S. Cascioferro, S. Plescia, M. A. Sabatino, G. Bombieri, F. Meneghetti, and G. Daidone, *ARKIVOC,* 2008, **xvi**, 130.
12. B. Maggio, D. Raffa, M. V. Raimondi, F. Plescia, M. L. Trincavelli, C. Martini, F. Meneghetti, L. Basile, S. Guccione, and G. Daidone, *Eur. J. Med. Chem.,* 2012, **54**, 709.
13. B. Maggio, D. Raffa, M. V. Raimondi, and G. Daidone, *Molecules*, 2013, **18**, 13096.
14. G. Daidone, B. Maggio, D. Raffa, and F. Meneghetti, *ARKIVOC*, 2014, **iv**, 80.
15. B. Maggio, G. Fontana, D. Raffa, F. Ferrante, and G. Daidone, *Heterocycles,* 2014, **89**, 83.
16. H. Katsuyama, H. Ono, and S. Watarai, JP Patent 49010744A, 1974 (*Chem. Abstr.,* 1974, **80**, 151136).
17. O. A. Miqdad, M. N. Abunada, H. M. Hassaneen, and A. S. M. Abu Samaha, *Int. J. Chem.,* 2011, **3**, 20.
18. A. Monteiro, L. M. Goncalves, and M. M. Santos Maria*, Eur. J. Med. Chem.,* 2014, **22**, 266.
19. T. Fukami, A. Kanatani, A. Ishihara, Y. Ishii, T. Takahashi, Y. Haga, T. Sakamoto, and T. Itoh, US Patent 20020188124A1, 2002, (*Chem. Abstr.,* 2002, **128**, 24705).
20. S. Guniz Kucukguzel and S. Senkardes, *Eur. J. Med. Chem.*, 2015, **97**, 786.
21. M. Vitale, C. Aiello, C. Fenaglio, F. Albicini, L. Emionite, R. Gangemi, and A. Balbi, *Pharmacol. Rep.,* 2013, **65**, 717.
22. L. Cecchi, F. Melani, G. Palazzino, and G. Filacchioni, *Il Farmaco*, 1985, **39**, 888.
23. J. F. Mc Elroy and R. J. Chorvat, US Patent 20070213302A1, 2007.
24. H. M. Eggenweiler and M. Wolf, WO Patent 2006018082A1, 2006.
25. M. J. Genin, C. Biles, J. Keiser Barb, S. M. Poppe, S. M. Swaney, W. G. Tarpley, Y. Yagi, and D. L.

Romero*, J. Med. Chem.*, 2000, **43**, 1034.

26. S. Plescia, G. Daidone, V. Sprio, E. Aiello, G. Dattolo, and G. Cirrincione, *J. Heterocycl. Chem.,* 1978, **15**, 1339.
27. F. Meneghetti, G. Bombieri, B. Maggio, and G. Daidone*, Acta Cryst.*, 2008, **E64**, 0863.
28. C. E. Newall and A. M. Eastham, *Can. J. Chem.*, 1961, **39**, 1752.
29. H. Mayr and M. Patz*, Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 938.