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One pot-like regiospecific access to 1-aryl-1*H*-pyrazol-3(2*H*)-one derivatives and evaluation of the anticancer activity

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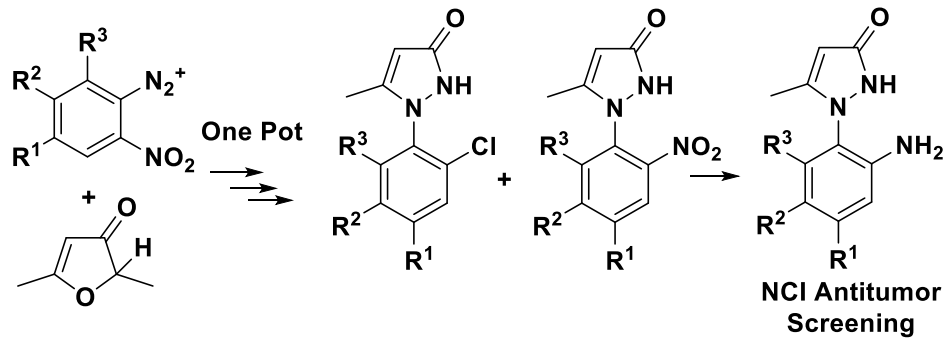
Abstract

A set of variously substituted 1-arylpyrazol-3-one derivatives, including the di-*ortho*-aryl substituted ones, was synthesized as new potential anticancer compounds. To fulfill this aim, herein a regiospecific synthesis was proposed utilizing a new revisited one pot procedure, starting from commercial anilines and easily accessible 2,5-dimethyl-furan-3-one. In the course of the sequential ordered steps, in some cases, a nitro group displacement by chlorine took place to a minor extent. The *in vitro* screening against the full panel of ~60 human cancer cell lines (NCI) showed a moderate, but promising selective antiproliferative activity against the UO31 renal tumor cell line, only in compounds with the introduction on the phenyl moiety of a -CF_3 or two Cl groups.



Keywords: Pyrazol-3-ones, antiproliferative activity, nitrogen heterocycles, regiospecific cyclization, NCI screening

Introduction

Cancer is a dangerous disease characterized by abnormal and uncontrolled cell growth that can occur in any part of the human body. Despite the excellent results obtained in the diagnosis and treatment of this disorder, the fight against cancer always remains an open challenge.[1]

From a medicinal chemistry point of view, much modern research pays particular attention to the discovery of new anticancer agents with optimal biological activities, and many efforts have been directed towards the synthesis of small molecules characterized by a heterocyclic scaffold. In this context, a large number of derivatives containing nitrogen atoms in the central core, (for example, pyridine and pyrimidine,[2-4] indole,[5] imidazole,[6] quinoline,[7,8] pyrazole and pyrrolizine[9,10] and pyrazolone derivatives,[11-13] and others[14-16]) have been described in literature and some of them have already been approved as therapeutics, thus showing great potential in the treatment of cancer.

Specifically, the pyrazolone heterocyclic ring is, and remains, an interesting scaffold for the development of new small molecules endowed with anticancer properties. Indeed, a wide number of pyrazolone-based compounds have been described in the literature, both as non-selective and selective (VEGFR and c-Met inhibitors) antiproliferative agents,[11,17] including some compounds demonstrating catalytic inhibition of human telomerase.[18]

In this context, we reported a revisited synthesis and the biological evaluation of a new series of substituted 1-aryl-pyrazol-3-one derivatives of type **1** (Figure 1). The research interest for these molecules relies on the assumption that these compounds represent a significant fragment of the related antiproliferative tricyclic pyrazolo[1,2-*a*]benzo[1,2,3,4]tetrazin-3-one analogues (PBTs), endowed with anticancer potential with GI_{50} values in the sub-micromolar range.[19,20] In an attempt to get insight into the minimal structural requirements, necessary to maintain the antiproliferative activity exhibited by the PBTs, we moved towards the synthesis of 1-aryl-pyrazol-3-one derivatives to evaluate how the increased conformational flexibility, jointly with the possible tautomeric equilibrium, could affect the activity in function of our selected functionalizations. Depending on the physical state, the chemical environment and the effect of substituents on the aryl moiety, including the possible formation of internal H-bonds, the derivatives **1** can exist in one of the two tautomeric forms (Figure 1).

After the synthetic campaign, the new series of 1-aryl-pyrazol-3-one molecules was submitted to the National Cancer Institute (NCI) for antiproliferative *in vitro* screening to evaluate the influence of the de-azo modification on the biological profile of the compounds.

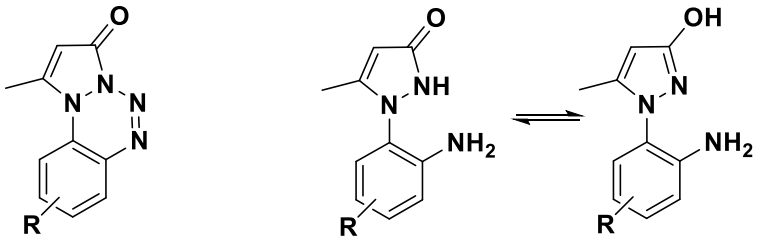
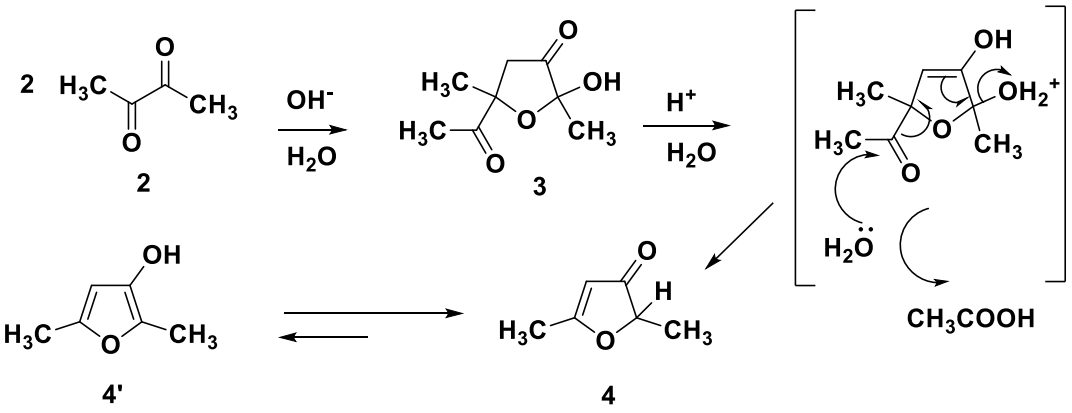


Figure 1. General structures of 1-aryl-pyrazol-3-one derivatives **1** and tautomeric possibilities of compounds synthesized in this study.

Moreover, compared with the previous synthesis described before,[19,20] herein, we report a revisited more advantageous preparation strategy to obtain the 1-aryl-pyrazol-3-one derivatives **1**, bearing also substitutions on the two *ortho*-aryl positions.

Results and Discussion

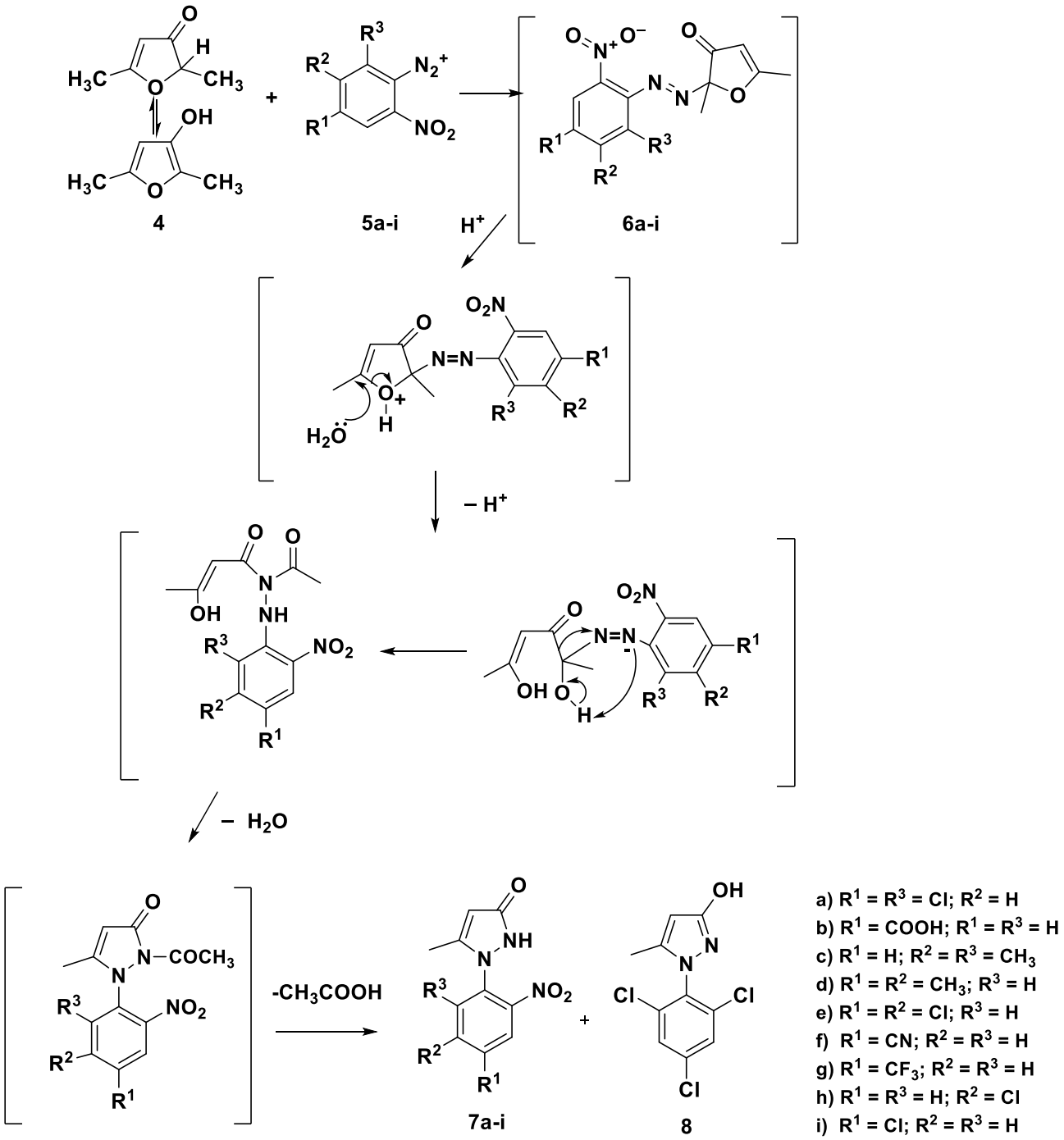
Chemistry. The general synthetic route for the preparation of the variously substituted 1-aryl-pyrazol-3-one derivatives started from the initial preparation of the furan-3-one intermediate **4**, as firstly described by Venturello and D'Alosio[21] (Scheme 1).



Scheme 1. Synthesis of the 2,5-dimethyl-furan-3-one.

In detail, the dimerization of the diacetyl **2** occurred in aqueous NaOH solution and afforded compound **3** in good yields. Subsequent heating, under aqueous acidic conditions, allowed the elimination of a molecule of acetic acid and the rearrangement of **3** into the dimethyl-furanone **4**, that can exist in equilibrium with the tautomeric form **4'**.

In the next steps, the commercially available 2-nitroanilines, converted into the corresponding diazonium salts **5a-i** upon treatment with sodium nitrite in concentrated hydrochloric acid, were coupled with the furanone **4** to give derivatives **7a-i** in appreciable yields after 24-48 h. The synthesis of the title derivatives accomplished here, with respect to the previous ones, was conducted in a one-pot manner, avoiding the isolation and purification of the aza-furanone intermediate **6a-i** or other intermediates. Thus, a step work-up economy is gained (Scheme 2), without appreciable variation of the overall yields.



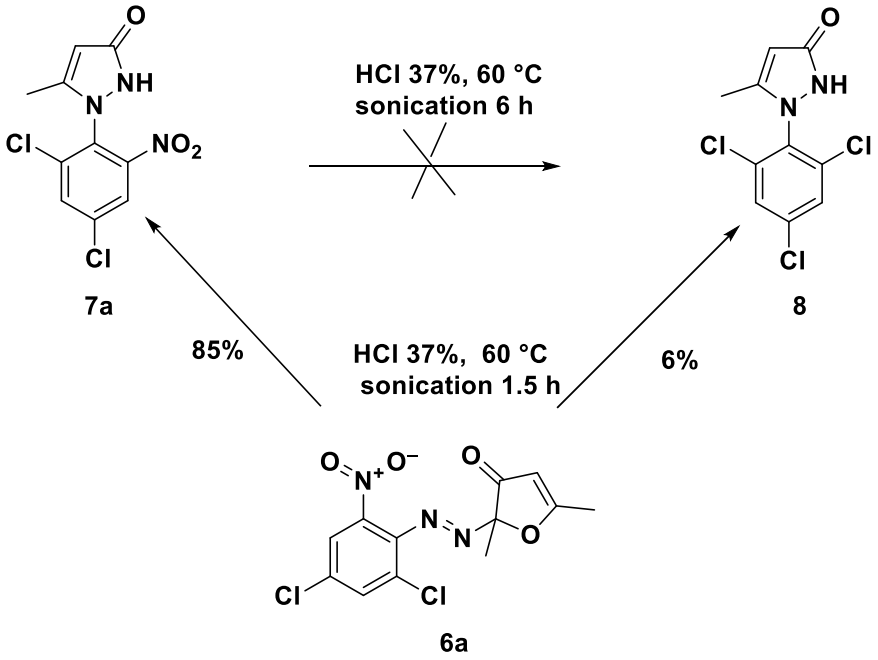
Scheme 2 Synthesis of 1-aryl-substituted-pyrazol-3-one derivatives **7a-i** and **8**.

Moreover, in this revisited procedure, we selected particularly **5a** and **5c** reactants, two di-*ortho* substituted nitro-anilines, R^3 = Cl and R^3 = Me groups, respectively, in order to evaluate how their electronic or steric effect could interfere with the entire rearrangement process. The isolation of the corresponding pyrazolones (**7a**, **7c**) demonstrated that these groups are well tolerated, involving only a slight decrease in yields.

The synthetic process above was carried out under strong acidic reaction and mild temperature conditions, necessary for all the ordered rearrangement process, and sometimes caused the appearance of a by-product. Specifically, in the reaction involving the aniline **5a,** we isolated, beside the the expected product, a new compound firstly detected by GC-MS spectroscopy, where the fragmentation pattern agreed with the typical isotope rate in agreement with a tri-Cl derivative of type **8** [presence of peaks M+ m/z 276 (100%), 278

(96.8%), 280 (31.5%)]. Further confirmations were furnished by ^{1}H and ^{13}C NMR spectroscopic data. Thus, in the course of the above sequential reaction carried out in a one pot procedure, a competitive nucleophilic substitution of the nitro group occurred, leading to the 1-(2,4,6-trichlorophenyl)-pyrazolone derivative **8**.

Although the observed nucleophilic substitution of the nitro group by chloride ion (Cl^-), could take place at different levels of the reaction sequence, it is reasonable to suppose that the pyrazolone **7a**, once formed, undergoes the nucleophilic attack by the chloride ion, in large excess in the reaction media, affording the 2,4,6-trichloro derivative **8** as a minor by-product (yield 10%). Based on our investigations until now performed, some clues support the following supposition: a) aryl-pyrazol-3-ones are particularly stable in strong acid media; b) pyrazolones possess basic nitrogen centers which can be protonated at low pH values and thus withdraw electron density on the aryl portion; and c) additional electronic effects exerted by the two chlorine atoms play a synergistic role in this sense. Therefore, we hypothesized that these combined electron-withdrawing effects, exerted by the combination of protonated pyrazolone portion and the di-chloro-aryl moiety, could favor the nitro group displacement. For this purpose, to validate our supposition, derivative **7a** was submitted to ultrasound stress in concentrated HCl (37%) for 6 h at 60 °C (Scheme 3). Notwithstanding all the favorable conditions above, after work-up, we recovered only the starting compound **7a**. Then, we considered the possibility that derivative **8** could originate during the rearrangement of the intermediate **6a**, not isolated in our one pot procedure. In this regard, we repeated the reaction in the classical procedure already described, by stopping the reaction at the aza-furanone step (by TLC monitoring). Thus, we isolated derivative **6a** and submitted it to rearrangement in HCl 37%. After 1.5 h of sonication at 60 °C, we were pleased to detect by GC-MS, beside the expected **7a** as major compound, a by-product corresponding to the identical fragmentation pattern as compound **8**.

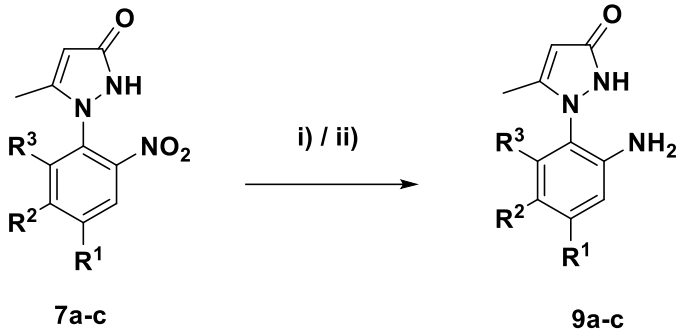


Scheme 3. Stability of **7a** after sonication under HCL 37% at 60 °C. Rearrangement of **6a** under HCL 37% at 60 °C promoted by sonication to furnish **7a**, along with minor extent of NO_2 replacement by chloride ion.

Due to the relevance of the amino group in biological interactions, we proceeded with the nitro conversion. Thus, to reach the 1-(*o*-aminoaryl)-pyrazol-3-one derivatives **9a-i,** we performed the reduction of the nitro

group with Fe/AcOH for the chlorine derivatives, and for the others by using classical catalytic conditions with Pd/C 10% in EtOH under H_2 (4 atm) in a Paar-like reactor (Scheme 4).

All compounds were fully characterized by ^1H, ^{13}C NMR, GC-MS, IR spectroscopy and microanalysis. All experimental data agreed with the proposed structures.



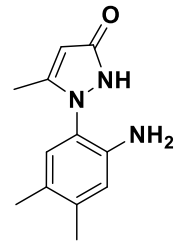
7a-c **9a-c**

i) Fe/AcOH, 60°C 3 h, for 7a ii) H_2/Pd on C, EtOH o.n. for 7b,c

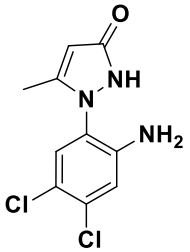
Scheme 4. Reduction of nitro derivatives **7a-c**

Preliminary *in vitro* studies on pyrazol-3-ones. The set of pyrazol-3-one intermediates synthesized, **7a-i**, **8**, and **9a-l**, were submitted to the National Cancer Institute for one dose screening (concentration of 10 μM) against the full NCI60 library, including sixty cell lines belonging to nine different tumor panels (leukemia, lung, renal, CNS, prostate, breast, melanoma, ovarian and colon).

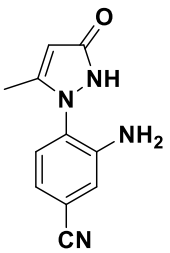
In general, the results for each tested compound are reported as the percent of growth (G%) of the treated cells when compared to the untreated control cells. This parameter strictly expresses the anticancer potential of the compound, indeed: for G%>100, the compound does not affect cancer cell proliferation; 0<G%<100, the compound inhibits the cell proliferation of a percentage given by 100-G%; if G%<0, the compound is cytotoxic and lethal for the cancer cells. Furthermore, to graphically appreciate the most sensitive panels/cell lines, a mean growth percent is provided. Among all, six pyrazol-3-one derivatives (**9d-g**[19], **9h,i**[20]) were selected and assayed against the NCI cancer cell panel (chemical structures reported in Figure 2).



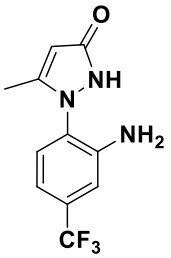
9d
(NSC752655)



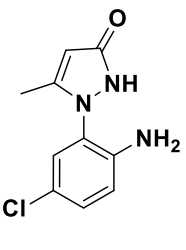
9e
(NSC752656)



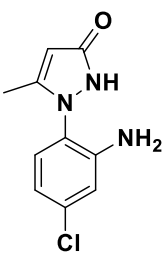
9f
(NSC752657)



9g
(NSC752658)



9h
(NSC752659)



9i
(NSC752660)

Figure 2. Pyrazol-3-ones (**9d-g**[19], **9h,i**[20]) selected for the NCI one dose screening assay.

In Table 1 the overall results obtained by the six selected compounds in the single-dose assay are reported: none of the tested compounds exhibited appreciable antiproliferative activity (expressed as G%) against the full NCI60 panels; indeed, all reported a mean G% higher than 100, meaning no reduction of cancer cell growth. Two compounds, **9e**[19] (NSC752656) and **9g**[19] (NSC752658), with the lowest mean G% (both 105.7) exhibited some modest results against UO-31 renal cancer cell line, with G% of 81.2 and 73.3, respectively.

Table 1 One Dose NCI screening of selected compounds (**9d-g**[19], **9h,i**[20])

Cell Panel	Cell Line	9d	9e	9f	9g	9h	9i
Leukemia	K-562	110.2	106	93.9	101	99.4	120.3
	MOLT-4	109.8	110.2	105.7	111.9	111.8	132.7
	RPMI-8226	103	104.7	102.4	103.5	105.7	103.4
	SR	115	90.6	96.2	107.4	96.6	112.8
Non-Small Cell Lung	A549/ATCC	107.5	100.2	102.8	97	104.5	102.1
	EKVX	105.4	101.5	106	93.9	116.5	126.9
	HOP-62	103.3	102.6	103.7	96.3	103.1	103.5
	HOP-92	90.1	112	120	111.1	113.6	111.2
	NCI-H23	104.4	104.2	100.1	96.6	106.5	96.2
	NCI-H322M	114.4	107.6	90.2	117.8	111.9	119.6
	NCI-H460	*N.T.**	*N.T.*	*N.T.*	115.6	112.7	114.5
	NCI-H522	*N.T.*	*N.T.*	*N.T.*	*N.T.*	*N.T.*	93.1
Colon	COLO 205	109.3	120.7	123	98.7	103	109.8
	HCT-116	108.5	106.7	116.1	90.5	95.6	104.7
	HCT-15	93.6	101.6	101.6	103.6	92.4	103.5
	HT29	111.6	99.9	108.7	117.8	102.4	95.9
	KM12	118.3	112.4	105.5	109.7	119	114.7
	SW-620	*N.T.*	*N.T.*	*N.T.*	*N.T.*	*N.T.*	105
Central Nervous System	SF-268	118.5	115.7	110.9	109.9	112.8	110.7
	SF-295	87.7	110.7	109.9	104.2	116.4	102.4
	SF-539	98.5	119.9	113.5	95.5	102.3	108.7
	SNB-19	101	112.2	101.6	139.5	112.2	102.1
	SNB-75	91	99.7	104.3	82.6	88.4	80.1
	U251	105.8	89	90.6	106.5	108	110.5
Melanoma	LOX IMVI	96.1	106.4	103.5	95.7	94.7	91.6
	MALME-3M	141.1	95.1	93.9	102.2	108.1	100
	M14	109.7	102.9	111.5	102.1	99.8	117.8

	MDA-MB-435	112.2	108.5	108.6	99.3	110.7	118
	SK-MEL-2	*N.T.*	*N.T.*	*N.T.*	*N.T.*	*N.T.*	103.8
	SK-MEL-28	111.1	108.8	118	109.6	115.1	112.3
	SK-MEL-5	111.9	108.5	110.8	107.1	112.3	107
	UACC-257	109.7	103.3	107.6	85.5	89.8	108.5
Ovarian	**IGROV1**	147.9	117.6	113.6	99	109.2	97.9
	OVCAR-3	127.2	104.1	119.9	105.8	123.5	111.4
	OVCAR-4	102.6	117.4	99.8	98.8	105.2	96.1
	OVCAR-5	107.5	102.7	100.9	116.4	110.5	115.1
	OVCAR-8	108.9	105.4	101.9	100.5	102.6	101.3
	NCI/ADR-RES	110.6	98.8	102.8	102.4	90.6	102.6
	SK-OV-3	98.1	102.2	98.4	89	98.6	97.9
Renal	**786-0**	104.6	112.2	106.2	102.1	103.8	120
	A498	*N.T.*	*N.T.*	87.1	131.7	119.6	122.4
	ACHN	106.2	111.6	106.9	106.9	100.5	108.9
	CAKI-1	101.7	99.4	135.7	*N.T.*	*N.T.*	92.6
	RXF 393	116.8	110.8	115.3	113.2	107.5	120.6
	SN12C	106.4	103.2	98.2	118.3	109.8	101.6
	TK-10	106.9	105.4	104.9	113.6	98.1	116.5
	UO-31	111.6	81.2	93.7	73.3	89.3	80.7
Prostate	**PC-3**	96.6	98.9	100.6	96.3	105.7	97.5
	DU-145	125.4	118.6	123.5	131.8	131.6	123.3
Breast	**MCF7**	98.5	97.7	99.3	100.3	100.5	106.2
	MDA-MB-231/ATCC	114.2	109	115.6	131.6	124.5	97.6
	HS 578T	*N.T.*	131.7	150.7	128.9	123.2	110.3
	BT-549	108.2	93.9	94.7	106	87.1	117.3
	T-47D	101.3	94.6	96.4	97.4	105.5	103.4
	MDA-MB-468	115.3	107.2	108.1	118.7	109.8	119.4
Mean G%		108.4	105.7	106.5	105.7	106.3	107.3

**N.T.: not tested against the cell line.*

In addition, although moderate, the derivative **9g** exhibited a growth inhibition (GI) against CNS tumor (SNB-75) and melanoma (UACC-257) cell lines, with 18% and 15% GI, respectively. Lastly, derivative **9i** deserves attention with 20% of inhibition growth, also against SNB-75 cells. These outcomes, even if modest, could be a basis for further developments in cell selectivity and decorations on new pyrazolone series.

Conclusions

Due to the increasing interest towards pyrazolone derivatives in the anticancer field, either as key intermediates or by incorporation in polycyclic heterocycles,[22] herein new insights into the synthesis and the antiproliferative properties of the 1-aryl-pyrazol-3-ones **7a-i**, **8** and **9a-i** are reported. In this light, we propose a more advantageous synthetic procedure, involving a step economy with respect to the previously reported methods in the literature, including the possibility to introduce functionalities in the hindered position of the 1-aryl moiety. In addition, this ordered regiospecific pathway can be alternatively performed under sonication stress, maintaining the N1-aryl isomer formation. Moreover, in some cases, depending on the effects of the substituents on the aryl portion, a nitro displacement by a chlorine could also be observed. Thus, six of the isolated compounds were selected for the NCI *in vitro* screening. Generally, none of them exhibited appreciable anticancer activity, with the exception of **9e** and **9g,** which reported a modest, but promising G% against the UO-31 renal cancer cell line. Deeper investigation will be conducted to investigate the reactivity of aryl-pyrazol-3-one derivatives, as well as analyze their potential biological activities, other than the anticancer one.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solution as specified below, using a Bruker AC-E series 200/300/400 MHz spectrometer. As internal reference, the residual peak of the solvent was calibrated, at d 7.26 and 2.50 ppm, respectively for $CDCl_3$ and DMSO-d_6 or using TMS as internal reference. The chemical shifts (δ) and coupling constants (J) are expressed in ppm and hertz respectively. "a" means "apparent" for close coupling constant and the number of equivalent group are indicated after the sign "x". Carbon attribution, namely C, CH, CH_2 and CH_3 were determined by ^{13}C and DEPT 135. IR spectra were recorded with bromoform on a NaCl window with a Bruker Alpha FT/IR spectrophotometer. All melting points were measured on a Sanyo-Gallenkamp capillary apparatus. Mass spectroscopy was performed using a GC-MS Shimadzu QP5050 with EI (75ev), equipped with a ZB5 Phenomenex 20m column. Column chromatography was performed with Merck silica gel 230 or 400Mesh ASTM. Thin layer chromatography was performed on precoated (0.25 mm) silica gel GF254 plates (Merck) and compounds were detected with 254/366 nM UV lamp. All reactions were carried out under hood and air exposed with commercially reagents purchased from Aldrich or Alfa Aesar and directly used. Sonication was performed with Transonic T460/H, ELMA (HF-Frequ 35KHz, A 1.30, F 50/60 Hz).

2-(2,4-Dichloro-6-nitro-phenylazo)-2,5-dimethyl-furan-3-one (**6a)**. To a suspension of 2,4-dichloro-6-nitro-phenylamine (414 mg, 2.00 mmol) in 5mL concentrated hydrochloric acid (37%), a solution of sodium nitrite (145 mg, 2.10 mmol) in water (1 mL) at 0 °C was added dropwise. The mixture was stirred for 2 h reaching room temperature (20°), until completion of diazotization. 2,5-Dimethyl-3-oxo-2,3-dihydrofuran[21] **4** (235 mg, 2.10 mmol) was slowly added dropwise with a syringe and the mixture was stirred at rt for 2 h. The so formed yellow solid was filtered, air dried, and purified by flash column chromatography using petroleum ether (40-60°C)/ EtOAc, 9:1. Collected fractions gave 535 mg of yellow powder of **6a** (yield 85%), recrystallized from EtOH mp 118-119°C, IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3084, 1711, 1601, 1538, 1438, 1384, 1344, 954, 897, 821. ^{1}H NMR (200MHz $CDCl_3$): δ_H: 1.66 (3H, s, CH_3), 2.42 (3H, s, CH_3), 5.80 (1H, s, CH furan), 8.26 (2H, s, CH x 2, aromatics). ^{13}C NMR ($CDCl_3$ 51 MHz) δ_C : 16.58 (CH_3), 18.49 (CH_3), 102.52 (CH furan), 107.83

(C furan), 124.32 (CH aromatic), 128.97 (C aromatic), 134.50 (CH aromatic), 138.00 (C aromatic), 140.62 (C aromatic), 141.52 (C aromatic), 191.42 (C aromatic), 193.44 (C aromatic). GC-MS: *M/z* (EI): (M^+: 329, 100%; 331, 60%) according to fragmentation pattern dichloro derivative. Anal. calcd for $C_{12}H_9Cl_2N_3O_4$ (329.0): C, 43.66; H, 2.75; N, 12.73. Found: C, 43.71; H, 2.69; N, 12.81.

Modified procedure for the preparation of substituted 5-methyl-1-(2-nitro-phenyl)-1*H*-pyrazol-3(2*H*)-one derivatives (7a-c). To a suspension of 2-nitro-anilines (5 mmol) in 20 mL concentrated hydrochloric acid (37%), a solution of sodium nitrite (0.370 mg, 5.38 mmol) in water (2 mL) was added dropwise at 0 °C. The mixture was stirred for 1-2 h, until completion of diazotization. In the case of the chloro-substituted aniline the reaction was carried out at room temperature (<20 °C). To this solution, freshly distilled 2,5-dimethyl-3-oxo-2,3-dihydrofuran[21] (620 mg, 5.5 mmol) was added dropwise and the mixture was stirred for 24 h at rt. The reaction time could be prolonged to longer than 24h, especially when withdrawing groups (R^1,R^2,R^3) were present on the aryl moiety (longer time was necessary for CF_3, Cl, and di-Cl groups). If a gummy residue was formed, an additional amount of conc. HCl (20 mL) was added, leaving the reaction mixture sufficient time for a filterable solid to form. Alternatively, the use of ultrasonic bath at 60°C for 1.5-2.5h, allowed quick formation of derivatives **7a-l** from the intermediate formed aza-furanone **6a-i** (by TLC monitoring). The crude residue was then poured onto ice water, treated with 30% aqueous solution of NaOH for the pH to reach 6.8. The so formed yellow-orange solid was collected by filtration, air dried and purified using silica gel chromatography with a CH_2Cl_2/EtOAc gradient as eluent, to give:

1-(2,4-Dichloro-6-nitro-phenyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one (7a). Recrystallized from EtOH as an orange-brown powder (yield 60%), mp 226-228 °C. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 2699, 2654, 2601, 2257, 1582, 1542, 1506, 1378, 1321, 1278, 1095, 1034, 874, 838, 759. ^{1}H NMR (200 MHz, $CDCl_3$): δ_H: 2.07 (3H, s, CH_3), 5.64 (1H, s, CH pyrazole), 8.32 (2H, CH x 2 aromatic, s), 10.07 (1H, s, NH). ^{13}C NMR ($CDCl_3$, 51 MHz) δ_C 10.94 (CH_3), 93.48 (CH pyrazole), 123.92 (CH aromatic), 128.80 (C aromatic), 129.04 (C aromatic), 133.78 (CH aromatic), 134.95 (C aromatic), 143.27 (C aromatic), 149.13 (C aromatic), 162.97 (C aromatic). GC-MS: *M/z* (EI): (M^+: 287, 100%; 289, 60%) according to dichloro derivative fragmentation pattern. Anal. calcd for $C_{10}H_7Cl_2N_3O_3$ (287.0): C, 47.18; H, 3.64; N, 15.41. Found: C, 47.27; H, 3.62; N, 15.47.

1-(4-Carboxy-2-nitro-phenyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one (7b). Recrystallized from EtOH as an orange powder (yield 70%): mp 259-261°C, over 264°C carbonization. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3400 broad, 2920, 2570, 1724, 1616, 1545, 1445, 1339, 1231, 840, 778. ^{1}H NMR (300 MHz, $DMSO_{d6}$) δ_H 2.23 (3H, s, CH_3), 5.69 (1H, s, CH pyrazole), 7.83 (1H, d, *J* 8.3 Hz, CH aromatic), 8.25 (1H, d, *J* 8.9 Hz, CH aromatic), 8.38 (1H, sa, CH aromatic), 10.18 (1H, broad s, NH). ^{13}C NMR ($DMSO_{d6}$, 75.5 MHz) δ_C 11.69 (CH_3), 95.09 (CH pyrazole), 125.75 (CH aromatic), 128.25 (CH aromatic), 130.46 (CH aromatic), 133.58 (C aromatic), 135.20 (C aromatic), 141.33 (C aromatic), 145.42 (C aromatic), 162.71 (C pyrazole), 165.07 (COOH). Anal. calcd for $C_{11}H_9N_3O_5$ (263.1): C, 50.20; H, 3.45; N, 15.96. Found: C, 50.05; H, 3.47; N, 16.01.

1-(2,3-Dimethyl-6-nitro-phenyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one (7c). Recrystallized from EtOH as an orange powder (yield 65%): mp 234-235 °C. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3500, 2550, 1579, 1524, 1501, 1384, 1364, 1320, 794, 785. ^{1}H NMR (200 MHz, $DMSO_{d6}$) δ_H: 1.94 (3H, s, CH_3), 1.96 (3H, s, CH_3), 2.40 (3H, s, CH_3), 5.58 (1H, s, CH pyrazole), 7.53 (1H, d, *J* 8.1 Hz, CH aromatic), 7.80 (1H, d, *J* 8.1 Hz, CH aromatic), 9.83 (1H, s, NH). ^{13}C NMR ($DMSO_{d6}$, 50.3 MHz) δ_C: 10.96 (CH_3), 13.75 (CH_3), 20.14 (CH_3), 92.04 (CH pyrazole), 121.51 (CH aromatic), 130.48 (C aromatic), , 130.5 4 (CH aromatic), 138.24 (C aromatic), 142.23 (C aromatic), 143.91 (C aromatic), 146.14 (C aromatic), 162.02 (C aromatic). GC-MS: *M/z* (EI): (M^+: 247). Anal. calcd for $C_{12}H_{13}Cl_2N_3O_3$ (247.1): C, 58.29; H, 5.30; N, 16.99. Found: C, 58.11; H, 5.32; N, 17.62.

5-Methyl-1-(2,4,6-trichloro-phenyl)-1*H*-pyrazol-3(2*H*)-one (8). Recrystallized from EtOH as a grey powder (yield 10%). mp 221-223 °C. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3082, 2957, 2921, 2696, 2650, 2595, 1579, 1550, 1507, 1444, 1374, 1317, 1277, 1090, 856, 840, 822, 804. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.01 (3H, s, CH_3), 5.60 (1H, s, CH pyrazole), 7.47 (2H, s, CH x 2 aromatics), 11.50 (1H, broad s, NH). ^{13}C NMR ($CDCl_3$, 101 MHz,): δ_C 11.10 (CH_3), 92.77 (CH pyrazole), 128.81 (CH x 2 aromatic), 132.99 (C aromatic), 136.28 (C aromatic), 136.57 (C x 2, aromatics), 142.84 (C aromatic), 163.64 (C aromatic). GC-MS: M+, *M/z*: 276 (100%), 278 (96.8%), 280 (31.5%). Anal. calcd for $C_{10}H_7Cl_3N_2O$ (276.0): C, 43.28; H, 2.54; N, 10.09. Found: C, 43.19; H, 2.52; N, 10.12.

General procedure for the preparation of substituted 1-(2-amino-phenyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one derivatives (9a-c)

Method A. Iron powder (670 mg, 12.0 mmol) was added to a solution of (3.4 mmol) in acetic acid (30 mL). The mixture was kept at 60 °C in a steam bath for 4 h (TLC monitoring). After cooling, the reaction mixture, was poured onto crushed ice and extracted with dichloromethane. The organic layer, dried over sodium sulfate and evaporated under reduced pressure, gave a residue, which was purified by chromatography using CH_2Cl_2/EtOAc mixture in gradient as eluant to give:

1-(2-Amino-4,6-dichloro-phenyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one (9a). Recrystallized from EtOH as white powder (yield 75%). mp 242-244 °C. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3478, 3337, 3088, 2953, 2600, 1618, 1533, 1504, 1427, 1325, 1282, 1072, 959, 823, 768. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.92 (3H, s, CH_3), 5.38 (2H, s, NH_2), 5.55 (1H, s, CH pyrazole), 6.79 (1H, d, *J* 2.4 Hz, CH aromatic), 6.82 (1H, d, *J* 2.4 Hz, CH aromatic), 9.80 (1H, s, NH). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 10.69 (CH_3), 91.94 (CH pyrazole), 113.17 (CH aromatic), 114.98 (CH aromatic), 120.08 (C aromatic), 134.00 (C x2, aromatic), 141.34 (C aromatic), 148.78 (C aromatic), 161.98 (C aromatic). GC-MS: *M/z* (EI) (M^+ 257, M^++2, 259 60%). Anal. calcd for $C_{10}H_9Cl_2N_3O$ (257.0): C, 46.53; H, 3.51; N, 16.28. Found: C, 46.71; H, 3.47; N, 16.32.

Method B. A solution in ethanol of nitro derivatives **7b,c** (42 mmol) was reduced overnight over 10% Pd on charcoal in a Parr like apparatus at 60 psi at 70 °C to room temperature. Removal of the catalyst and evaporation of the solvent under reduced pressure gave a residue which was purified by flash chromatography using a CH_2Cl_2/EtOAc mixture in gradient as eluant.

1-(2-Amino-4-carboxy-phenyl)-5-methyl-1H-pyrazol-3(2H)-one (9b). Recrystallized from ethanol (yield 90%) as yellow-orange solid. mp 257- 259 °C. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3400 broad, 2552, 1696, 1625, 1578, 1518, 1470, 1389, 1321, 1200, 767. ^{1}H NMR (400 MHz, $DMSO_{d6}$): δ_H 2.08 (3H, s, CH_3), 4.68 (4H, broad s, NH_2, NH, COOH), 5.68 (1H, s, CH pyrazole), 7.19 (1H, d, *J* 8.1 Hz, CH aromatic), 7.25 (1H, dd, *J* 8.1, 1.8 Hz, CH aromatic), 7.53 (1H, d, *J* 1.8 Hz, CH aromatic). ^{13}C NMR ($DMSO_{d6}$, 101 MHz): δ_C 12.04 (CH_3), 93.13 (CH pyrazole), 118.42 (CH x2 aromatic), 127.54 (CH aromatic), 127.98 (CH aromatic), 131.69 (C aromatic), 142.60 (C aromatic), 143.26 (C aromatic), 161.57 (C aromatic), 167.39 (COOH). Anal. calcd for $C_{11}H_{11}N_3O_3$ (233.1): C, 56.65; H, 4.75; N, 18.02. Found: C, 56.48; H, 4.77; N, 18.09.

1-(2-Amino-5,6-dimethyl-phenyl)-5-methyl-1*H*-pyrazol-3(2*H*)-one (9c). Recrystallized from EtOH as white powder (yield 98%). mp 238-240 °C. IR (bromoform, NaCl cell window, νmax, cm^{-1}): 3450, 3425, 3340, 3212, 3133, 2950, 2695, 1627,1567, 1537, 1504, 1324, 1037, 806. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 1.86 (3H, s, CH_3), 1.96 (3H, s, CH_3), 2.19 (3H, s, CH_3), 3.49 (3H, broad s, NH2 and NH), 5.54 (1H, s, CH pyrazole), 6.5 (1H, d, *J* 8.1 Hz, CH aromatic), 6.99 (1H, d, *J* 8.1 Hz, CH aromatic). ^{13}C NMR ($CDCl_3$,62.8Hz): δ_C 10.48 (CH_3), 13.41 (CH_3), 13.41 (CH_3), 18.78 (CH_3), 90.81 (CH pyrazole), 112.62 (CH aromatic), 123.31 (C aromatic), 126.22 (C aromatic), 130.80 (CH aromatic), 135.67 (C aromatic), 141.96 (C aromatic), 142.21 (C aromatic), 162.82 (C aromatic). GC-

MS: *M/z* (EI) (M^+ 217). Anal. calcd for $C_{12}H_{15}N_3O$ (217.1): C, 66.34; H, 6.96; N, 19.34. Found: C, 66.14; H, 6.92; N, 19.29.

Characterization of derivatives **7d-i** and **9d-i** confirmed data as previously reported.[19,20] and exhibited melting points identical or varying within +/- 1°C with respect the original samples.

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Supplementary Material

Copies of the ^1H, ^{13}C NMR, and FT/IR spectra of the new compounds are reported in the Supplementary Material file associated with this manuscript.

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