Article

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Microwave-assisted synthesis of diversely substituted quinoline-based dihydropyridopyrimidine and dihydropyrazolopyridine hybrids

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ABSTRACT: An efficient, catalyst-free and one-pot three-component procedure for the synthesis of novel and nitrogen rich dihydropyrido[2,3-*d*]pyrimidines and dihydro-1*H*-pyrazolo[3,4-*b*]pyridines bearing a quinoline pharmacophore fragment is provided. Reactions proceeded in DMF under microwave irradiation of three-component mixtures of formyl-quinoline derivatives, primary heterocyclic amines and cyclic 1,3-diketones. Interestingly, when conventional heating at reflux was used for the starting 5-amino-1-phenylpyrazole, the corresponding aromatized pyrazolopyridines were obtained as the main products. Single crystal X-ray analysis confirmed unequivocally the structure of both the dihydro- and aromatized products.

INTRODUCTION

The pyrimidine core has been widely studied due to its presence in numerous natural products and in structurally diverse synthetic derivatives.[1] In particular, pyridopyrimidines and pyrazolopyridines have received considerable attention over the past years, due to their wide ranging biological activities, mainly as antifolates,[2] showing related antiproliferative properties such as anticancer,[3] anti-inflammatory,[4] antimicrobial[5] or antihistaminic[6] agents. Figure 1 shows several examples of bioactive compound with these core structures. The BPIPP **1** was identified as novel potent inhibitor of cyclic nucleotide biosynthesis and a promising lead compound for treatment of diarrhea and other diseases.[7] Other linear tricyclic derivatives like pyrimido[4,5-*b*][1,6]naphthyridines **2**[8] and pyrazolo[3,4-*b*]pyridines **3**,[9] exhibited significant anticancer activity, while the BPIPP analogues **4** were evaluated against fifteen *Mycobacterium* spp strains, some of them displaying remarkable antimycobacterial activity.[10]

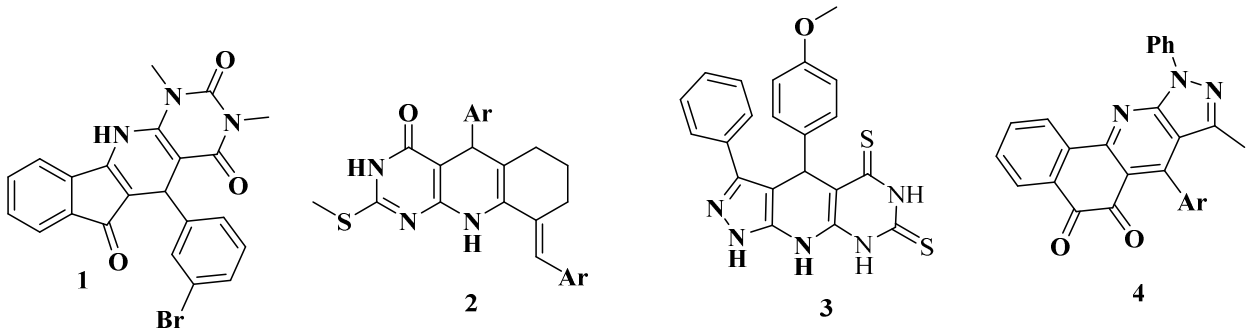


Figure 1. Examples of bioactive pyrido[2,3-*d*]pyrimidine and pyrazolo[3,4-*b*]pyridine derivatives.

On the other hand, quinolines and their oxo-derivatives are another important class of heterocyclic compounds in chemistry, biology, medicine and in applied pharmacy. Examples of some bioactive quinolone based derivatives are displayed in Figure 2: thus, the 2-alkoxyquinoline and *N*-alkylated quinolinone derivatives **5** and **6** were evaluated as potent and selective cannabinoid-2 receptor ligands through a bioisosteric approach,[11] while, the quinoline-2-one hybrids **7-10** are considered as antitumor agents,[12a,b] including a series of chalcones **9** and benzimidazoloquinolin-2-ones **10** that we have recently reported.[12c,d]

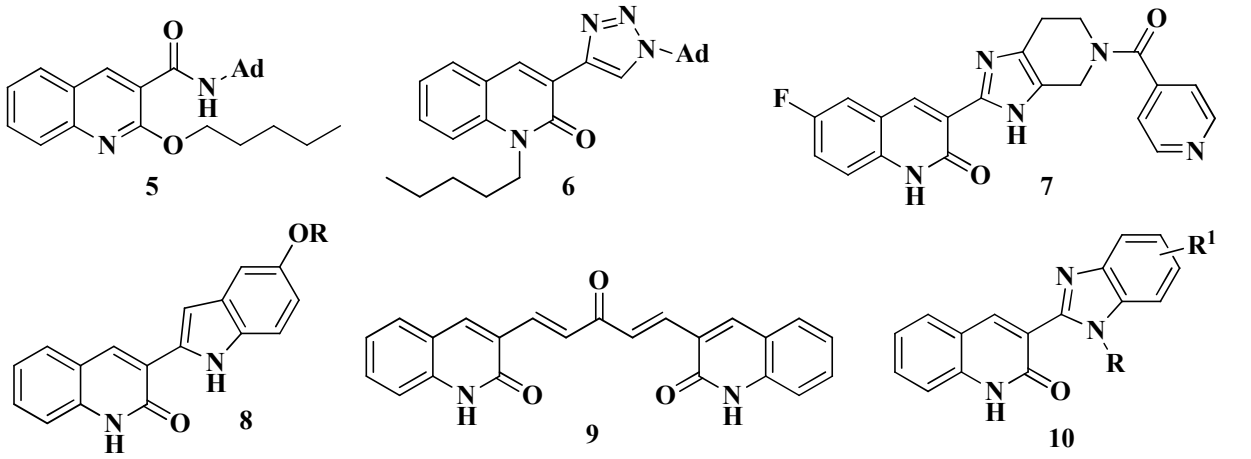


Figure 2. Examples of bioactive quinoline-hybrids.

Focusing on the synthetic methodologies, the multicomponent reactions (MCRs) have become a powerful tool for accessing a vast number of synthetic and pharmaceutically relevant compounds. The main advantages of the MRCs are high reaction rates, selectivity and efficiency, as well as, low costs, high variability, high bond forming efficiency, simplicity and environmentally friendly properties.[13] In the same way, microwave assisted organic synthesis (MAOS) is also a powerful tool, because it can decrease reaction times, improve yields and purity of the final compounds, through the control of parameters such as temperature, pressure and power irradiation.[14] Therefore, the combination of both MCR and (MAOS) strategies offers a rapid and cost efficient synthetic strategy to prepare diverse natural occurring and synthetic products of great interest to fine chemical and drug discovery endeavors.

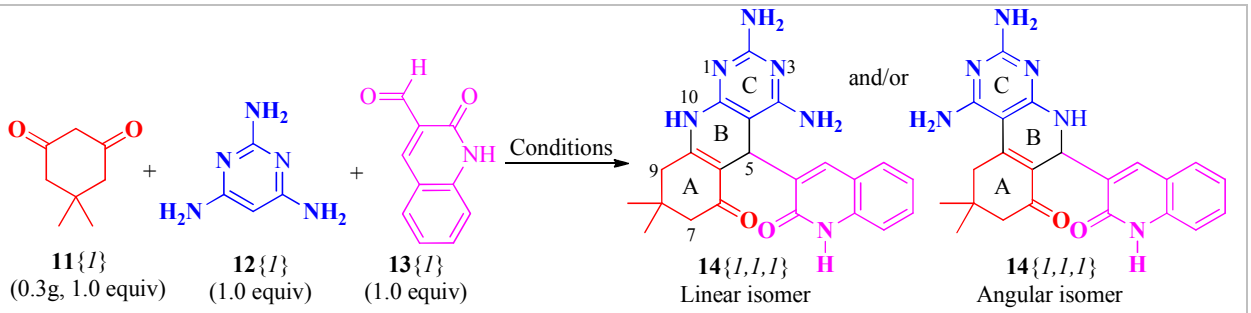
In connection with our continuing interest in the synthesis of bioactive nitrogen-containing heterocyclic compounds bearing the quinoline pharmacophore fragment,[12c,15] herein we report a versatile and efficient method for the preparation of diversely substituted quinoline-hybrids, with dihydropyrido[2,3-*d*]pyrimidines and dihydro-1*H*-pyrazolo[3,4-*b*]pyridines, by means of microwave irradiation (MWI) assisted, one-pot, three-component reaction with various heterocyclic primary amines, cyclic 1,3-diketones and diverse 3-formyl *N*- / *O*-alkyl-quinolines.

RESULTS AND DISCUSSION

According to previous related reports on the dihydropyrido[2,3-*d*]pyrimidine core construction,[7,8,16] we visualized that parent frameworks could be synthesized bearing the outstanding quinoline pharmacophore in their structures. In order to obtain the desired products *via* a three-component protocol, several synthetic approaches were tested involving diverse solvents, along with conventional heating or microwave irradiation, in an effort to find optimal

reaction conditions for our transformations. In all cases, reactions were carried out between dimedone **11**{*1*}, 2,4,6-triaminopyrimidine **12**{*1*} and 3-formyl-2-oxo-quinoline **13**{*1*} as a model reaction (see Table 1). Initially, stirring of an equimolar mixture of the above three-components in ethanol (5 mL) at reflux, did not afford the product after 20 h of heating (TLC monitoring). The limitation was associated to the low solubility of both pyrimidine **12**{*1*} and aldehyde **13**{*1*} in this solvent (Table 1, entry 1). The problem was resolved with the use of DMF instead of ethanol, and heating the ternary mixture in DMF at reflux gave the expected product **14**{*1,1,1*} (linear isomer), albeit in only 38% isolated yield after 20 h of reflux (Table 1, entry 2). In order to improve the reaction yield, our model reaction was carried out by applying MWI in DMF (1 mL) as the solvent at different temperatures (Table 1, entries 3-5). From the best result, the linear isomer dihydropyridopyrimidine **14**{*1,1,1*} was isolated in 82% yield after 8 min of irradiation at 130 °C (Table 1, entry 5).

Table 1. Optimization of the reaction conditions for the three-component synthesis of dihydropyridopyrimidine **14**{*1,1,1*}



11{*1*} (0.3g, 1.0 equiv) **12**{*1*} (1.0 equiv) **13**{*1*} (1.0 equiv) **14**{*1,1,1*} **14**{*1,1,1*}

Entry	Conditions	Time	Yield (%)
1	ethanol, reflux	20 h	-
2	DMF, reflux	20 h	38
3	DMF, MWI, 70 °C	16 min	43
4	DMF, MWI, 100 °C	12 min	56
5	DMF, MWI, 130 °C	8 min	82

6	DMF, MWI, 150 °C	8 min	75[a]

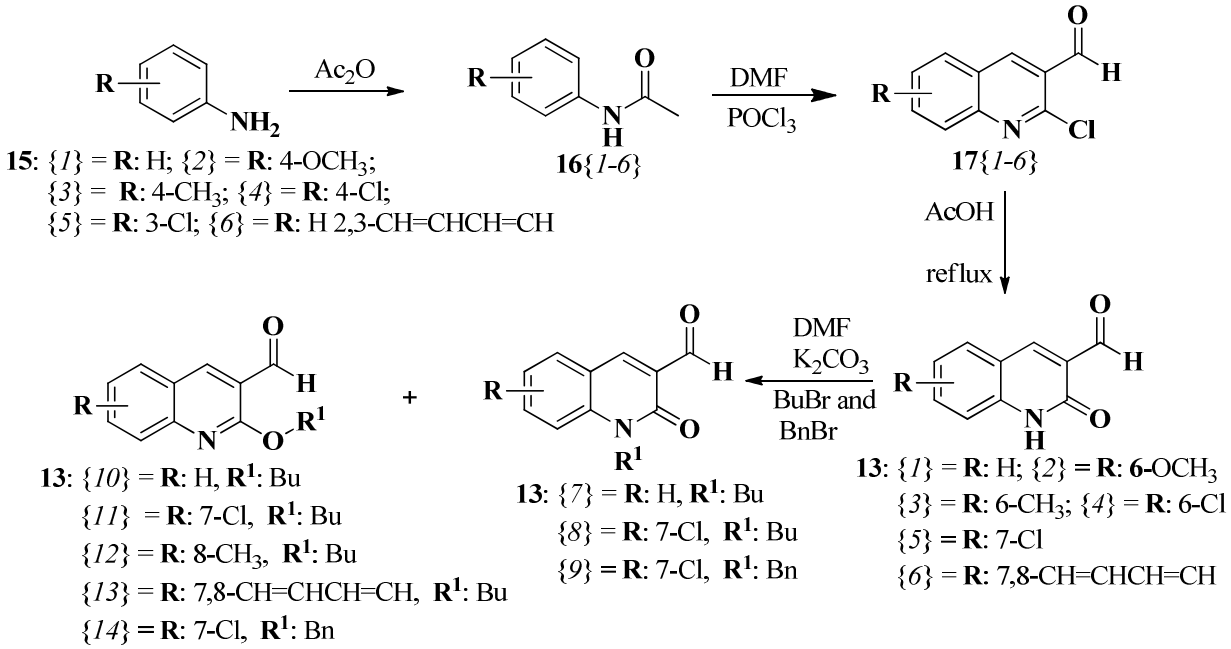
[a]Some decomposition products accompanied this assay.

The linear structure of the obtained compound **14**{*1,1,1*} was ascertained by IR, 1D and 2D NMR and MS spectrometric analysis. The most relevant spectroscopic features in the IR spectrum correspond to the presence of three NH and NH_2 absorption broad bands at 3404, 3340 and 3170 cm^{-1} and two C=O absorption bands at 1645 and 1614 cm^{-1}. In the ^1H NMR spectrum, among others, the presence of two singlets at 0.99 and 1.04 ppm (integrating for 3H each), two doublets at 1.95 and 2.18 ppm (integrating for 1H each) and a singlet overlapped with the DMSO at 2.5 ppm. These five signals are assigned to the dimedone residue. Presence of a singlet at 5.06 ppm integrating for 1H assigned to H-5 proton, along with a broad singlet at 9.36 ppm corresponding to the 10-NH functionality agree with the formation of the dihydropyrido[2,3-*d*]pyrimidine framework **14**{*1,1,1*}. Additionally, NOESY experiments confirmed the formation of the linear isomer instead of its angular regioisomers. In this case spatial NOE correlations between H-9 and 10-NH protons, as well as, 4-NH_2 and H-5 protons were clearly observed, among others. In the ^{13}C NMR spectrum the presence, among others, of six aliphatic signals (i.e. two CH_3, one Cq, two CH_2 and one CH) also agrees with the proposed structure for **14**{*1,1,1*}. In particular, the 5-CH signal at 27.9 ppm confirms the formation of the hydropyridinic moiety in such structure. A molecular ion peak with *m/z* 402, also confirmed in such a structure.

In order to evaluate the reproducibility and application scope of this approach, a diversely substituted chemset of 3-formyl-2-oxo-quinolines **13**{*1-9*} and 3-formyl-2-alkoxy-quinolines **13**{*10-14*} was synthesized. Firstly, the 2-chloroquinoline-3-carbaldehydes **17**{*1-6*} were obtained from a Meth-Cohn type methodology mediated by the Vilsmeier-Haack (DMF + $POCl_3$) reagent.[17] Subsequent treatment of compounds **17** with AcOH at reflux led to isolation of

3-formyl-2-oxo-quinolines **13**{*1-6*}. The alkylation and benzylation of compounds **13**{*1-6*} with 1-bromobutane and benzyl bromide, respectively, afforded the remaining *N*-/*O*-butyl- and *N*-/*O*-benzyl-quinolines **13**{*7-9*} and **13**{*10-14*}, respectively, Scheme 1 and Figure 3.

Scheme 1. General procedure for the synthesis of the starting formyl-quinolines 13.



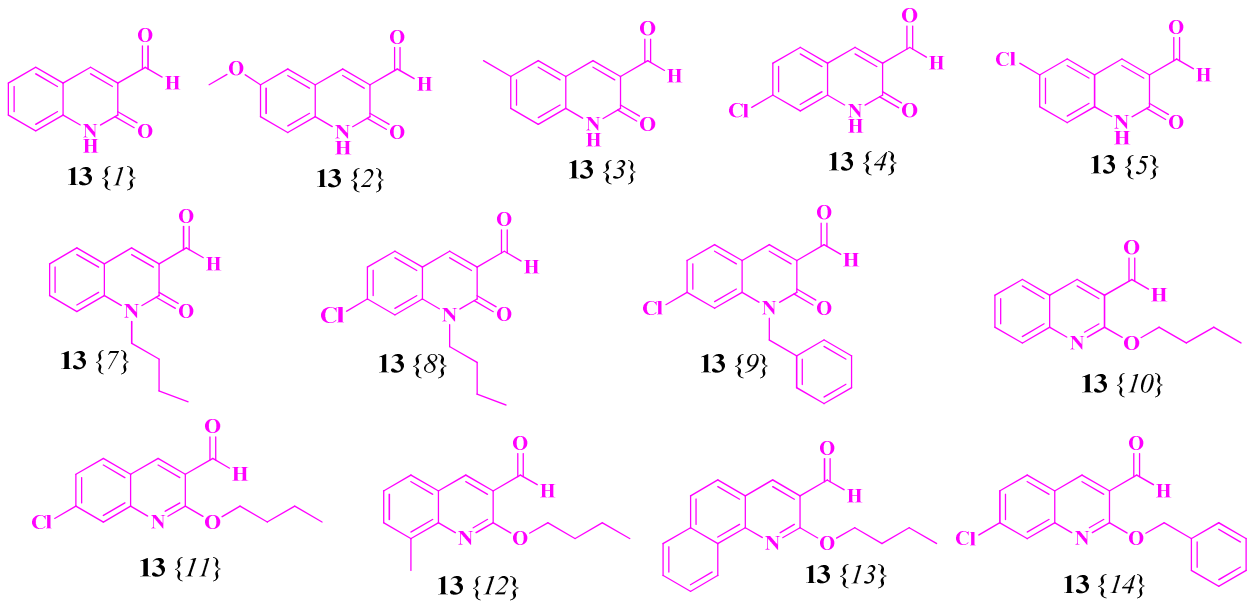


Figure 3. Diversely substituted 3-formyl-quinolines **13** employed as reagents for the synthesis of the title compounds **14**.

In addition to the above results, and in an effort to increase diversity in this three-component strategy a chemset of cyclic 1,3-diketones **11**{*1-3*} and primary heterocyclic amines **12**{*1-4*} was considered, Figure 4. It was also necessary to prepare aminopyrimidines **12**{*2-3*} and aminopyrazole **12**{*4*} because these reagents are not commercially available.[18,19]

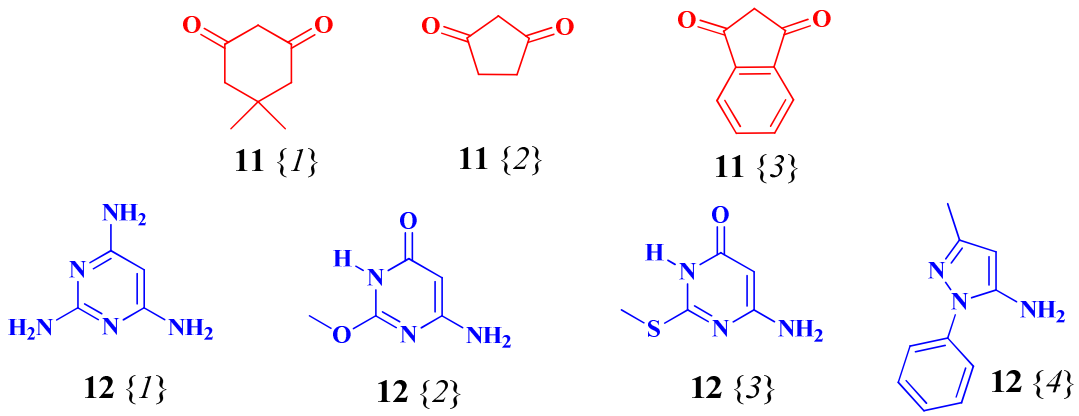
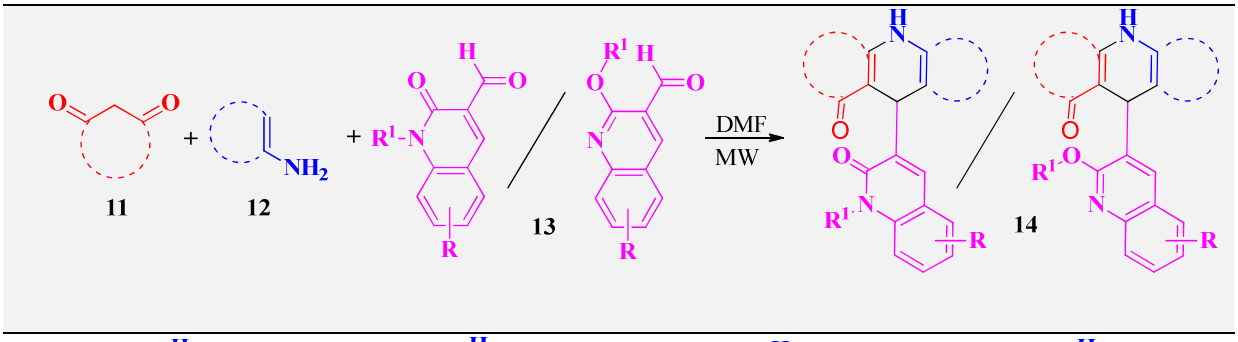
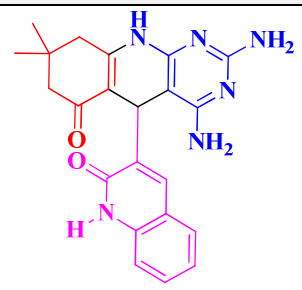


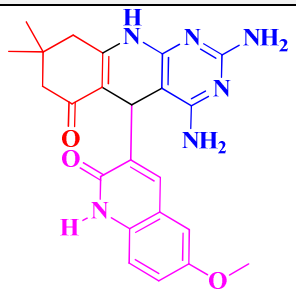
Figure 4. Diverse chemset of cyclic 1,3-diketones **11** and heterocyclic primary amines **12** to be used in the MCR.

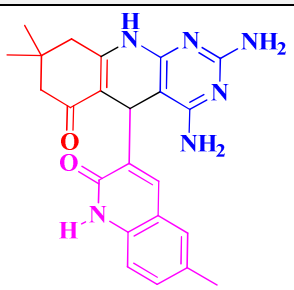
It is noteworthy that reaction between diketones **11**{*1-3*}, aminopyrimidines **12**{*1-3*}, and 3-formyl-quinolines **13**{*1-14*} under the MWI established reaction conditions, satisfactorily led the expected products **14** in all cases. Reactions proceeded in similar way than that for **14**{*1,1,1*} with yields in the range of 68-86% (Table 2). Their structures were also confirmed by a complete analytical and spectroscopic analysis.

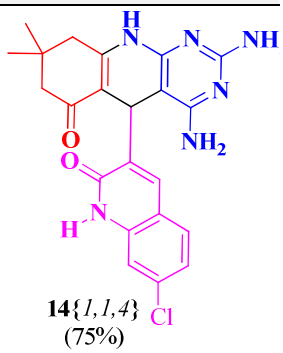
Table 2. Three-component MAOS of the dihydropyrido[2,3-*d*]pyrimidines **14**.

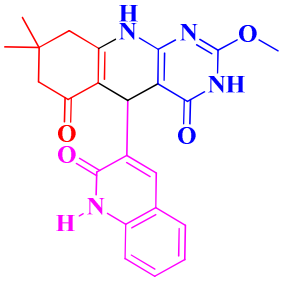


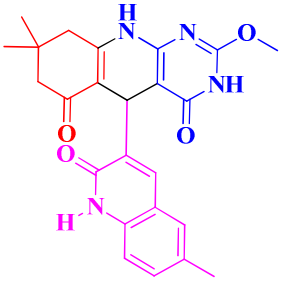

14{*1,1,1*}
(82%)

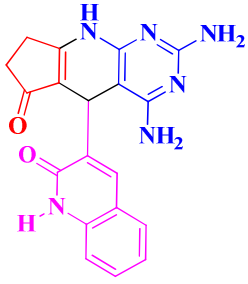

14{*1,1,2*}
(73%)

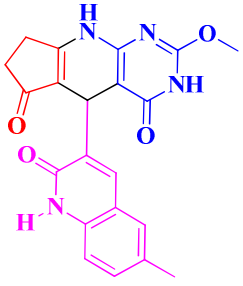

14{*1,1,3*}
(69%)


14{*1,1,4*}
(75%)

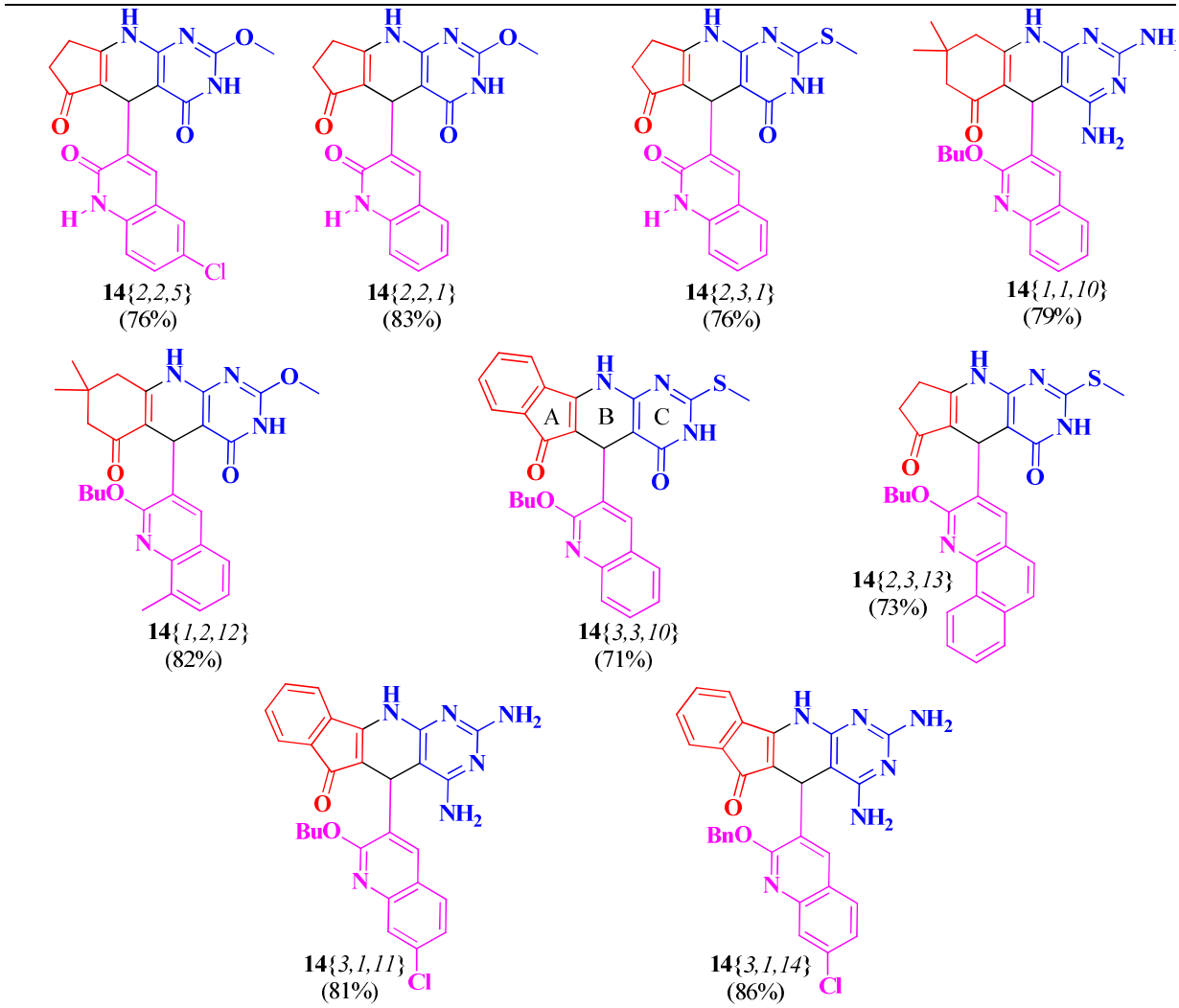

14{*1,2,1*}
(80%)


14{*1,2,3*}
(81%)


14{*2,1,1*}
(84%)


14{*2,2,3*}
(68%)

Continued Table 2



Additionally, single crystals of compound **14**{*3,3,10*} suitable for X-ray diffraction were grown from a solution of DMF at ambient temperature, Figure 5. (See also Supporting Information). The X-ray molecular structure confirm the formation of the linear dihydropyrido[2,3-*d*]pyrimidine framework in **14** without ambiguity.

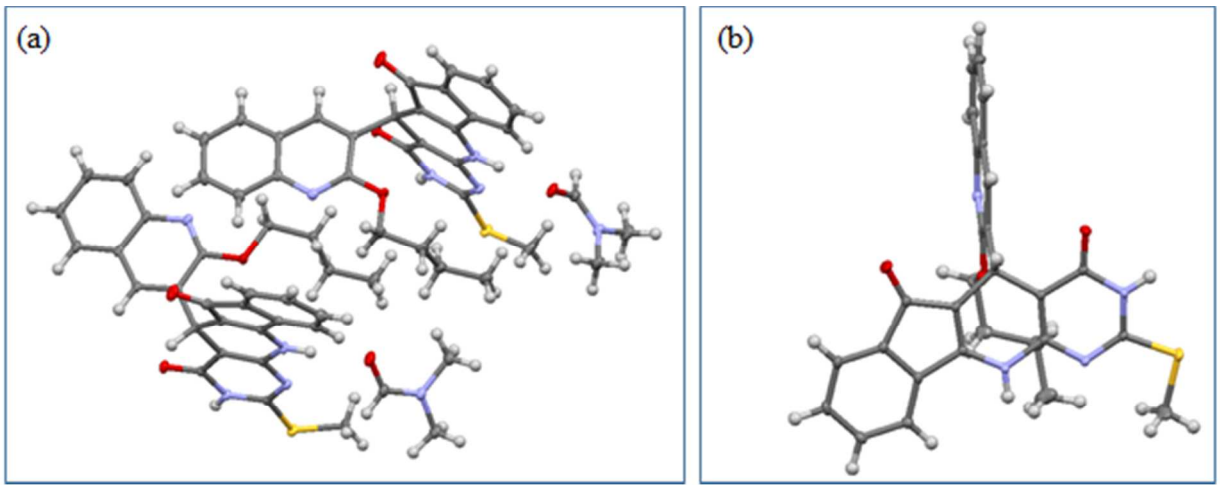
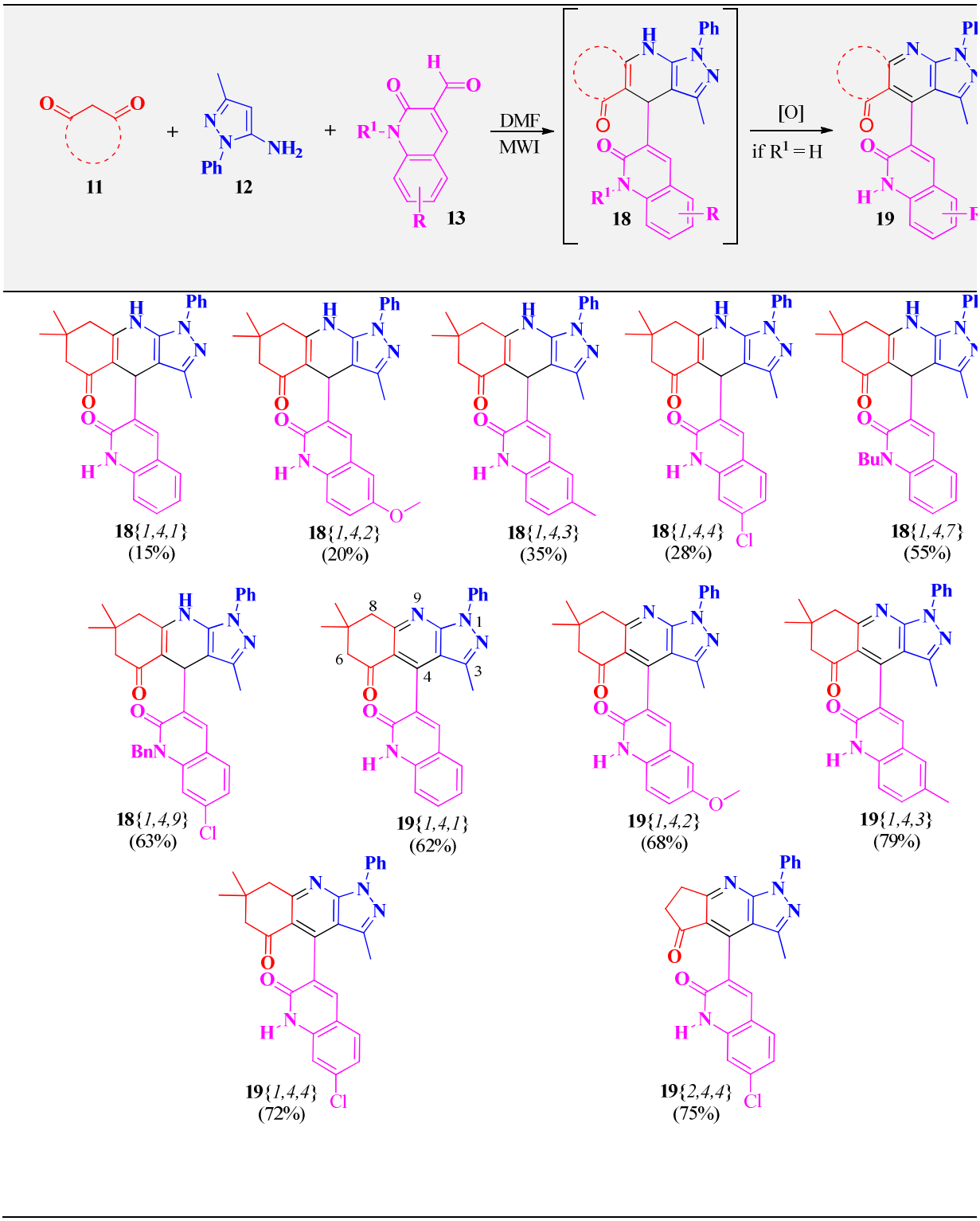


Figure 5. Displacement ellipsoid plots (40% probability level) of (a) the asymmetric unit for the racemic **14**{*3,3,10*} DMF solvate. (b) Molecular structure of (*S*)-steroisomer (Mercury 3.8).

In order to expand the generality of this procedure toward primary heterocyclic amines, we subjected the previously synthesized 5-amino-3-methyl-1-phenyl-1*H*-pyrazol **12**{*4*}, Figure 4, as well as selected diketones **11** and fomyl-quinolines **13** to our established methodology. This approach afforded the expected dihydro-1*H*-pyrazolo[3,4-*b*]pyridine frameworks **18** in the range of 15-63% isolated yield, Table 3. It is worth mentioning that these yields are relatively lower than those obtained from the aminopyrimidines **12**{*1-3*}.

Table 3. Three-component MAOS of dihydro-1*H*-pyrazolo[3,4-*b*]pyridines **18** and their aromatized analogues **19**.



We assume that the modest yields of compounds **18** are associated with decomposition of the starting amine **12**{*4*} under the reaction conditions, detected during the course of the reaction

and confirmed by controlled experiments. In an attempt to improve the reaction yields of the pyrazolo-derivatives **18**, we decided to return to the earlier reaction conditions in DMF at reflux under conventional heating (i.e. entry 2, Table 1. Reflux by 8 h), specifically for the assays with aminopyrazole **12**{*4*}. Interestingly, the reaction proceeded with less decomposition of amine **12**{*4*} and better yields (62-79%) were achieved by employing this approach although the isolated products corresponded to the aromatized pyrazolo[3,4-*b*]pyridine analogues **19** instead of their expected dihydro-derivative ones **18**, specifically, when R^1 = H (see compounds **19**{*1,4,1*} through **19**{*2,2,4*} in Table 2 and compare with their analogues **18**). We suggest that in this case the reactions proceed through the initial formation of the dihydro-derivatives **18**, and under the reaction conditions undergo subsequent aromatization, leading to the isolated products **19**. Spectroscopic analysis support the proposed structures for **19**.

In order to confirm the suggested aromatization process, we were able to obtain single crystals of the corresponding isolated product **19**{*1,4,1*} from DMF. The analysis by X-ray diffraction showed the formation of the aromatized pyrazolo[3,4-*b*]pyridine framework in products **19**, Figure 6. (See also Supporting Information).

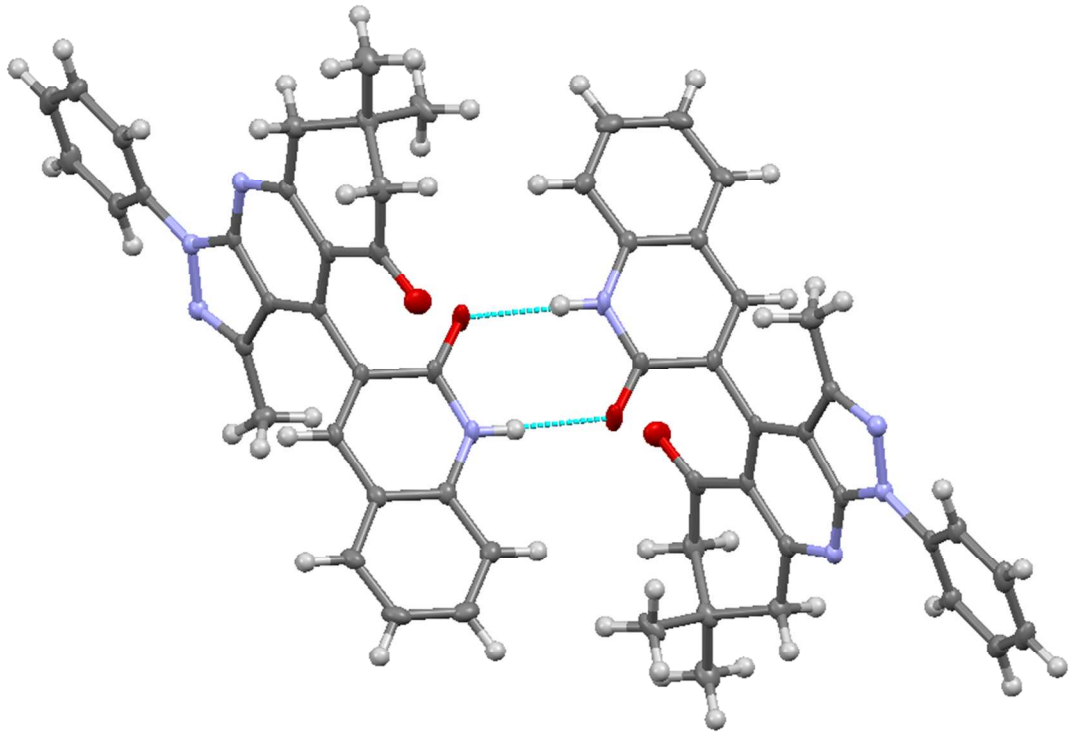


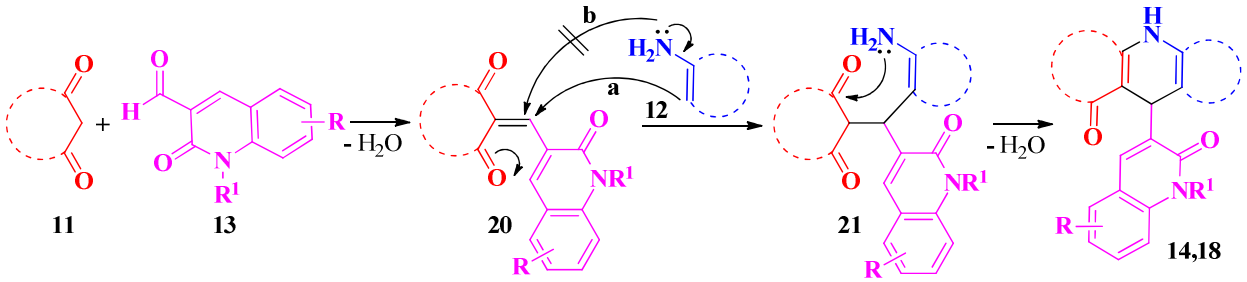
Figure 6. Displacement ellipsoid plot (40% probability level) corresponding to the dimeric unit formed by a cyclic intermolecular hydrogen bonding of two asymmetric units of compound **19**{*1,4,1*} (Mercury 3.8).

It should be noted that the aromatization process was exclusively observed for the aminopyrazole **12**{*4*} derivatives but not for those obtained from aminopyrimidines **12**{*1-3*}. Apparently the starting formyl-quinolines **13**{*1-4*} R^1 = H (but not their *N*-alkylated analogues **13**{*7,9*}), under the refluxing reaction conditions (not MWI), are contributing factors for this transformation.

A similar process toward aromatized pyridine rings has previously been reported in the literature when aminopyrazoles were used instead of aminopyrimidines.[20]

According to these results, it may be suggested that the synthesis of the linear dihydroderivatives **14** and **18** starts with *in situ* formation of the α,β-insaturated intermediate **20** *via* an aldol condensation between ketone **11** and formyl-quinoline **13**. Subsequently, a Michael addition of the amine **12** to intermediate **20** furnishes the keto-amine **21** (route **a**). Next, an intramolecular attack of the amino group onto the carbonyl functionality on structure **21** leads the isolated linear products **14** and **18** with elimination of a molecule of water, as can be depicted in Scheme 2. It is noteworthy that the Michael addition of **12** towards **20** proceeds exclusively by the β-carbon atom of **12** (route **a**), but not by the amino group (route **b**). This latter addition would have led to angular isomeric products instead of the obtained linear ones.

Scheme 2. Proposed mechanistic sequence for the formation of the linear dihydro-derivatives **14** and **18**.



Taking into account that our synthetized compounds are hybrids of biological interest,[2,12,15] some selected structures were subjected to preliminary antitumor assays. Initially, the structures of all new compounds **14, 18** and **19** were submitted to the Developmental Therapeutics Program (DTP) at National Cancer Institute (NCI), Bethesda, MD (USA), for evaluation of their viability as anticancer agents against different human cell lines.

Then, the output from the single dose screening was analyzed by the COMPARE program[21] (data not shown), and compounds **14**{*1,1,2*}. (NSC: 783179/1), **14**{*1,1,3*} (NSC: 783180/1), **14**{*1,2,1*} (NSC: 784183/1), **14**{*1,2,3*} (**NSC:** 784181/1), **14**{*1,2,12*} (**NSC**: 784184/1),), **14**{*3,3,10*} (NSC: 784185/1), **18**{*1,4,1*} (NSC: 784178/1), **18**{*1,4,2*} (NSC: 784179/1), **18**{*1,4,4*} (NSC: 784180/1), were selected by the Drug Evaluation Branch of the (NCI) for *in vitro* antiproliferative screening against a panel of 60 cancer cell lines at a single dose of 10 μM. The 60 cell panel is derived from nine different cancer types: *Leukemia*, *Non-Small Cell Lung*, *Melanoma*, *Colon*, *CNS*, *Ovary*, *Renal*, *Breast* and *Prostate* cancers. Results for each compound were reported as a mean graph of the growth percent (GP) of the treated cells and they are illustrated in Table 4 for the more sensitive lines. The synthesized compounds displayed moderate to low activity in the *in vitro* screening on all tested cancer cell lines, being compound **18**{*1,4,4*} the most active of the series against NCI-H522 cell line (*Non-Small Cell Lung* panel) with a GP = 40.23%, followed by T-47D (*Breast* panel) with a GP = 59.00%. Compounds **14**{*1,1,2*}, **14**{*1,1,3*} and **14**{*1,2,3*} showed moderate activity against NCI-H522 (*Non-Small Cell Lung* panel) with GP = 64.10%, 66.32 and 61.15, respectively. The remaining compounds in Table 4 showed less activity with an average growth percent of 65.23 - 89.76.

Table 4. Range and mean growth % of NCI human cancer cell lines treated with some selected compounds **14** and **18** at one-dose 10 μM.

Compound	**Some selected cell lines of the 60 cell lines panel assayed by the NCI**			
	NSC: number	Mean GP	Most sensitive cell lines	GP of the most sensitive cell lines
14{*1,1,2*}.	NSC: 783179/1	96.19	NCI-H522 (*Non-Small Cell Lung*)	64.10
			A498 (*Renal*)	71.02

14{*1,1,3*}	NSC: 783180/1	95.84	NCI-H522 (*Non-Small Cell Lung*)	66.32
			UACC-257 (*Melanoma*)	76.33
14{*1,2,1*}	NSC: 784183/1	99.08	NCI-H522 (*Non-Small Cell Lung*)	73.75
			TK-10 (*Renal*)	76.57
14{*1,2,3*}	NSC: 784181/1	99.55	NCI-H522 (*Non-Small Cell Lung*)	61.15
			TK-10 (*Colon*)	80.05
14{*1,2,12*}	NSC: 784184/1	102.78	NCI-H522 (*Non-Small Cell Lung*)	77.32
			SNB-75 (*CNS*)	87.16
14{*3,3,10*}	NSC: 784185/1	97.05	NCI-H226 (*Non-Small Cell Lung*)	72.97
			MALME-3M (*Melanoma*)	76.23
18{*1,4,1*}	NSC: 784178/1	99.17	A549/ATCC (*Non-Small Cell Lung*)	75.42
			NCI-H226 (*Non-Small Cell Lung*)	74.85
18{*1,4,2*}	NSC: 784179/1	97.84	PC-3 (*Prostate*)	84.19
			T-47D (*Breast*)	86.18
18{*1,4,4*}	NSC: 186.07	784180/	NCI-H522 (*Non-Small Cell Lung*)	40.23
			T-47D (*Breast*)	59.00

In summary, we have developed an efficient, catalyst-free, three component approach for the synthesis of novel and diversely substituted linear dihydropyridopyrimidines **14** and dihydropyrazolopyridines **18** mediated by MWI. When conventional heating at reflux was used in the case of the starting aminopyrazole **12**{*4*}, aromatized pyrazolopyridines **19** were obtained

as the main products, specifically, for formyl-quinolones **13** having R^1 = H. This process should involve the previous formation of the corresponding hydro-derivatives **18**. Subsequent oxidation/aromatization is apparently influenced by the mode of heating and the structure of the formyl-quinoline used. Considering that overall three starting materials are stoichiometrically consumed in the course of the reactions forming three new bonds (i.e. 2 x C-C and a C-N) in only one-step, and with water as the only by-products, the present approach brings together outstanding bond-forming efficiency and environmentally friendly quality. Preliminary *in vitro* antitumor assays at a single dose of 10 μM showed that the evaluated compounds displayed moderate to low activity against all tested cancer cell lines.

EXPERIMENTAL PROCEDURES

General: Melting points were measured using a Stuart SMP3 melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IRAffinity-1 Spectrophotometer in KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrophotometer operating at 400 MHz and 100 MHz respectively, using DMSO-d_6 as solvent and tetramethylsilane as internal standard. Mass spectra were run on a SHIMADZU-GCMS 2010-DI-2010 spectrometer (equipped with a direct inlet probe) operating at 70 eV. High resolution Mass spectra were run on a Waters Micromass AutoSpec-Ultima spectrometer (equipped with a direct inlet probe) operating at 70 eV. Microanalyses were performed on an Agilent CHNS elemental analyzer and the values are within ± 0.4% of the theoretical values. Single-crystal X-ray data for compounds **14**{*3,3,10*} and **19**{*1,4,1*} were collected at room temperature (298 K) on a Bruker D8 Venture (APEX 3) diffractometer and deposited at Cambridge Crystallographic Data Center. Microwave experiments were carried out using a focused microwave reactor (CEM Discover

TM), with dynamic method. TLC analyses were performed on silica gel aluminum plates (Merck 60 F_{254}) and spots visualized with ultra-violet irradiation. The starting cyclic diketones **11**{*1-3*}, triamine **12**{*1*}, precursors and reagents for the synthesis of amines **12**{*2-4*} and formyl-quinolines **13**{*1-14*}, and the required solvents were purchased from Sigma-Aldrich, Fluka and Merck (analytical grade reagent), and were used without further purification.

General procedure for the synthesis of the dihydropyrido[2,3-*d*]pyrimidines 14 and dihydro-1*H*-pyrazolo[3,4-*b*]pyridines 18 under MWI. A mixture of equimolar amounts of the aminopyrimidine **12**{*1-3*} or 5-aminopyrazol **12**{*4*} (0.05 mmol), cyclic diketone **11**{*1-3*} (0.05 mmol) and the formyl-quinoline **13**{*1-14*} (0.05 mmol) in DMF (1.0 mL), was subjected to MWI for 8-20 min at 125-135 °C, 250 W of power and 30 PSI of pressure. After finish (TLC monitoring), the reaction mixture was allowed to cool to room temperature, the solids formed were collected by filtration, washed with ethanol (2 x 3 mL) and dried in air. No further purification was required.

General procedure for the synthesis of the pyrazolo[3,4-*b*]pyridines 19 under conventional heating at reflux. A dry 25 mL one-neck flask was charged with 5-aminopyrazole **12**{*4*} (0.05 mmol), cyclic diketones **11**{*1,2*} (0.05 mmol), formyl-quinolines **13**{*1,4*} (0.05 mmol) and DMF (5 mL). The reaction mixture was stirred at reflux for 8 h (TLC monitoring), and the solid formed was filtered under reduced pressure and washed repeatedly with warm ethanol. No further purification was required.

ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures, compound characterization data, ^{1}H, ^{13}C NMR spectra for all products and X-ray diffraction data. This material is available free of charge via the Internet at http://pubs.acs.org

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Microwave-assisted synthesis of diversely substituted quinoline-based dihydropyridopyrimidine and dihydropyrazolopyridine hybrids

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