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# Polysubstituted pyrazoles, part 6. Synthesis of some 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazol-3-carbonyl derivatives linked to nitrogenous heterocyclic ring systems as potential antitumor agents ☆,☆☆

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ABSTRACT

The synthesis of two novel series of 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazoles linked to either polysubstituted 1*H*-pyrazole counterparts through a carbonyl bridge, or to some biologically-active nitrogenous heterocycles by an amide linker, is described. Ten of the newly synthesized compounds were selected by the National Cancer Institute (NCI) in vitro disease-oriented antitumor screening to be evaluated for their antitumor activity. The most active six compounds **2**, **3**, **6**, **7**, **13** and **14** revealed a significant broad spectrum of antitumor potential against most of the tested subpanel tumor cell lines at the $GI_{50}$ and TGI levels, together with a mild cytotoxic ($LC_{50}$) activity. The pyrazolinedione analog **7** displayed remarkable growth inhibition and cytostatic effects ($GI_{50}$ and TGI MG-MID values 0.67 and 53.8 μM, respectively). Compounds **13** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.08, 30.9 and 93.3 μM) and **14** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.36, 8.78 and 69.3 μM, respectively) proved to be the most active antitumor members identified in this study.



## 1. Introduction

Several lines of evidence support the view that chemotherapy has become one of the most significant treatment modalities in cancer management. Nevertheless, from early on in the development of chemotherapy, it was realized that the window in which the dose range is both efficacious and safe is small. Consequently, the principal obstacles to the clinical efficacy of chemotherapy remain their possible toxicity to normal tissues of the body, beside the development of cellular drug resistance especially to conventional anticancer agents.[1,2] As a result, the design and discovery of non-traditional, efficient and safe chemical classes of agents are prime targets in contemporary medicinal chemistry. Over the past two decades, pyrazole-containing compounds have received considerable attention owing to their diverse chemotherapeutic potentials including versatile antineoplastic activities. Literature survey revealed that some pyrazoles have been implemented as antileukemic,[3–5] antitumor[6–9] and anti-proliferative[10,11] agents, beside their capability to exert remarkable anticancer effects through inhibiting different types of enzymes that play important roles in cell division.[12–14]

As a part of an ongoing research program devoted to the synthesis and characterization of different heterocyclic ring systems endowed with potential chemotherapeutic activities,[15–28] particular attention has been given to the pronounced anticancer activity of several new 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxylic acid hydrazide derivatives having the general formula **A** (Fig. 1).[18,19] According to the protocol of the NCI's in vitro disease-oriented antitumor screen unit, Bethesda, Maryland, USA,[29–31] seven analogs exhibited potential antitumor activity, often associated with high or moderate selectivity for certain human tumor cell lines. As a consequence, three compounds incorporating the above-mentioned pyrazole counterpart together with either 1,3,4-oxadiazole or 1,2,4-triazole moieties were further selected by the NCI's biological committee for subsequent confirmatory screening procedures in order to optimize the exposure time to the agent and to define its possible mechanism of action,[32] which would be useful for determining the necessity of the subsequent in vivo studies.

Prompted by the above-mentioned results, it was rationalized to further optimize this chemical series (4-hydroxy-1*H*-pyrazoles) by exploring additional modifications at position 3 of the pyrazole

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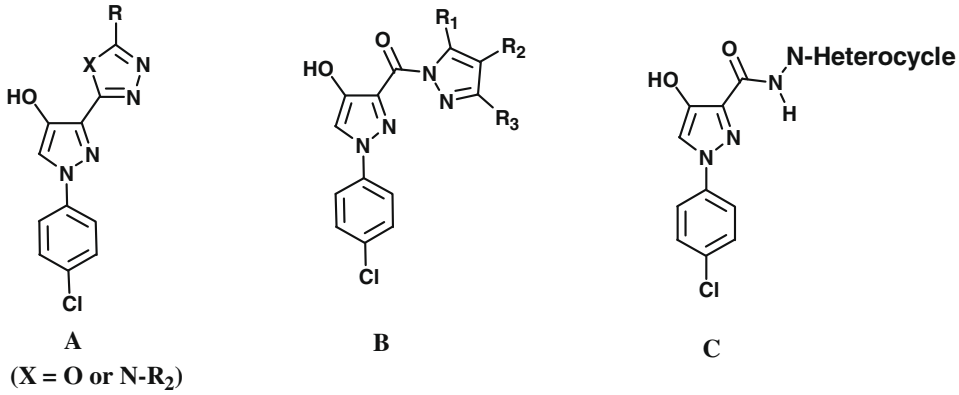



**Figure 1.**

ring, keeping the same 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole scaffold responsible for the anticancer profile unchanged. Structure modifications suggested in the present investigation focussed mainly on studying the effect of linking various polysubstituted pyrazole counterparts to position 3 by a carbonyl bridge (Formula **B**; Fig. 1). The substitution pattern of the target compounds include various functionalities that would act as hydrogen-bond forming centers, such as the carbonyl, amide, amino, cyano, hydroxy and carbethoxy groups. In addition, variation in the nature and size of other substituents was also attempted, as it would offer variable electronic, lipophilic and steric environment that would influence the targeted biological activity. It was considered also worthwhile to extend structure variation by attaching some biologically-active nitrogenous heterocycles such as indole, isoindole, quinazoline, pyrrole, β-carboline at position 3 of the main pyrazole moiety through an amide linker (Formula **C**; Fig. 1). Interest in such substitution profile stemmed from the reported contribution of different linear and semi-linear fused di- and tricyclic ring systems as a scaffold for DNA intercalation and kinase inhibition.[33–35] These combinations were suggested in an attempt to investigate the possible synergistic influence of such structure hybridizations on the anticipated activity, hoping to discover a new lead structure that would have a significant antitumor potential at very small concentrations.

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of the target compounds **2**–**21** is depicted in Schemes 1–3. In Scheme 1, the starting compound 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxylic acid hydrazide **1**[36] was allowed to react with ethyl acetoacetate in ethanol to produce the pyrazolinone derivative **2**. Condensing **1** with various substituted diketones in glacial acetic acid gave rise to the corresponding 3,5-disubstituted pyrazoles **3**–**6**. Whereas, heating the same acid hydrazide **1** with diethyl malonate at 200 °C afforded the targeted pyrazolidine-3,5-dione **7**. On the other hand, reacting **1** with various substituted diethyl malonate in the presence of sodium methoxide resulted in the formation of the 4-substituted-pyrazolidine-3,5-diones **8**–**10**. Condensing **1** with 2-acetylbutyrolactone in refluxing bromobenzene afforded the 5-(2-hydroxyethyl)-4-methyl-3-oxopyrazol-2-yl derivative **11**. Analogously, compound **12** was synthesized by direct fusion of **1** with ethyl cyclopentanone-2-carboxylate. Whereas, reacting the same starting compound **1** with 2-acetyl-1-tetralone afforded the targeted 3-methyl-4,5-dihydronaphtho[1,2-*c*]pyrazol-2-yl analog **13**.

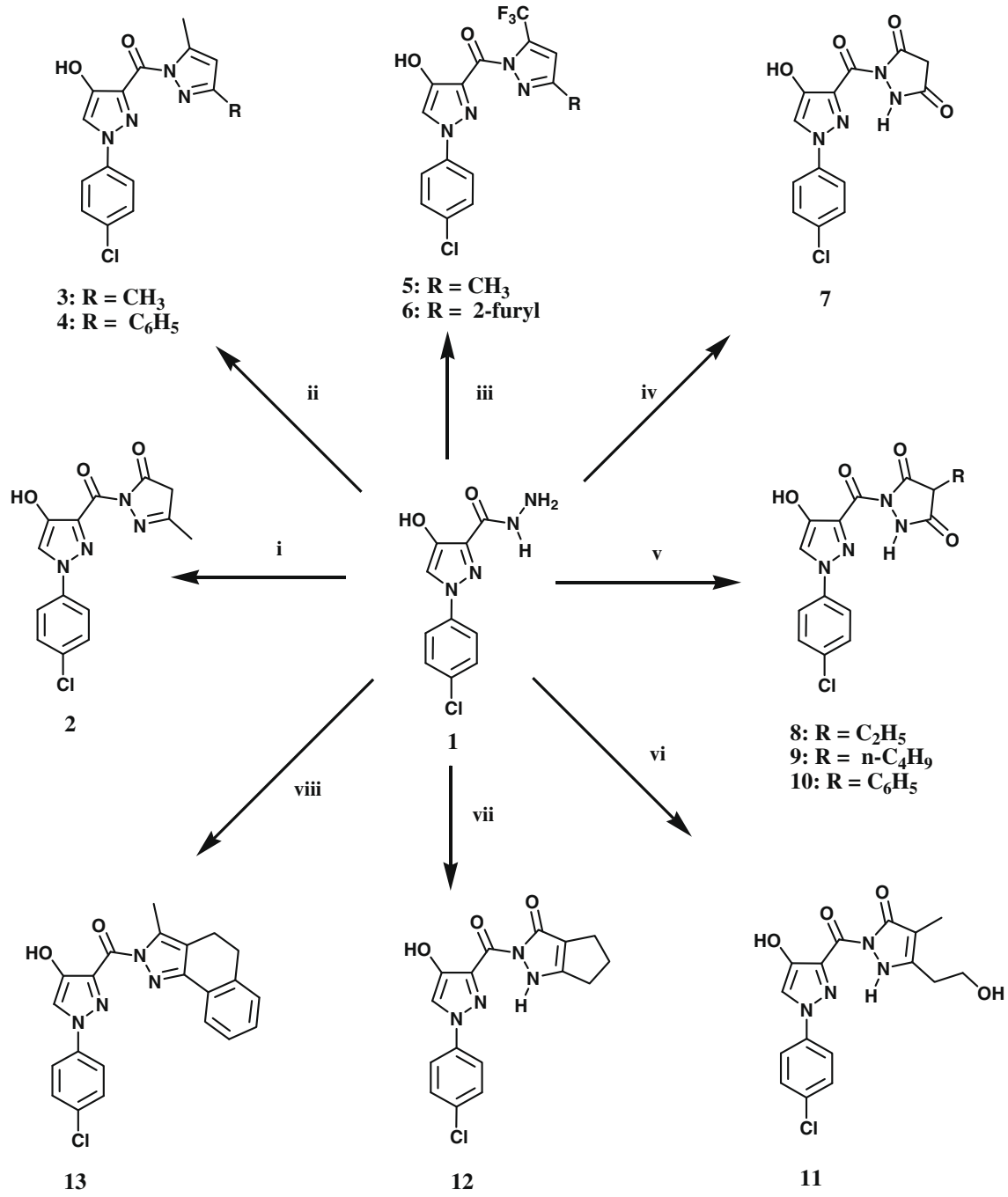
Referring to Scheme 2, condensation of **1** with ethoxymethylenemalononitrile or ethyl ethoxymethylenecyanoacetate in absolute ethanol, in the presence of anhydrous potassium carbonate resulted in the formation of the corresponding 5-amino-4-cyano-1*H*-pyrazol-1-yl **14** and the 5-amino-4-ethoxycarbonyl-1*H*-pyrazol-1-yl **15** derivatives, respectively. Analogously, when **1** was reacted with diethyl ethoxymethylenemalonate in refluxing acetonitrile, in the presence of anhydrous potassium carbonate, the targeted 4-ethoxycarbonyl-5-hydroxy-1*H*-pyrazole analog **16** was obtained.

On the other hand, Scheme 3 illustrates the preparation of the target compounds **17**–**21** in which the 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole scaffold was linked to different nitrogenous heterocyclic ring systems through a carboxamido functionality. Thus, the starting acid hydrazide **1** was allowed to react with maleic anhydride in glacial acetic acid in the presence of anhydrous sodium acetate to produce *N*-(2,4-dioxo-2,5-dihydropyrrol-1-yl)-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxamide **17**. Whereas, heating **1** with phthalic anhydride in boiling xylene afforded the *N*-(1,3-dioxo-1,3-dihydroisoindol-2-yl) analog **18**. Moreover, when **1** was reacted with acetanthranil[37] or 3,4-dihydropyrano[3,4-*b*]indol-1(9*H*)-one[38] in glacial acetic acid, the corresponding 1-(4-chlorophenyl)-4-hydroxy-*N*-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)-1*H*-pyrazole-3-carboxamide **19** and the *N*-(3,4-dihydro-1-oxo-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl) analogs **20**, respectively, were obtained. Finally, condensing the acid hydrazide **1** with isatin in glacial acetic acid yielded the azomethine derivative **21**.

### 2.2. In vitro antitumor activity

#### 2.2.1. Primary in vitro one-dose assay

Out of the newly synthesized compounds, ten derivatives **2**–**7**, **13**–**15** and **18**; were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro antitumor activity. Effective one-dose assay has been added to the NCI 60 Cell screen in order to increase compound throughput and reduce data turnaround time to suppliers while maintaining efficient identification of active compounds.[29–31] All the selected compounds were tested initially at a single high dose (10 μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would progress to the five-dose screen. The threshold inhibition criteria for progression to the five-dose screen was designed to efficiently capture compounds with anti-proliferative activity and is based on careful analysis of historical Development Therapeutic Program (DTP) screening data. The data are reported as a mean-graph of the percent growth of treated cells, and presented

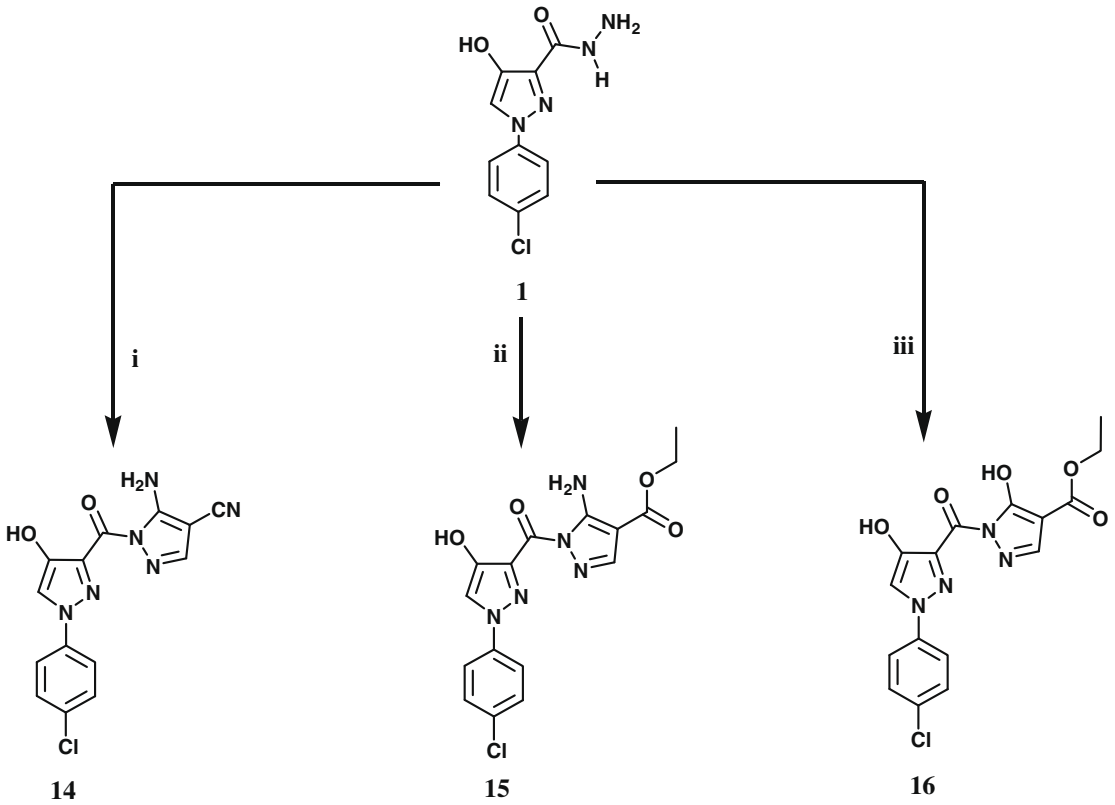



**Scheme 1.** Reagents and reaction conditions: (i) $CH_3COCH_2COOC_2H_5$, abs ethanol, reflux 10 h; (ii) $CH_3COCH_2COR$, gl. acetic acid, reflux 8–12 h; (iii) $CF_3COCH_2COR$, gl. acetic acid, reflux 8–12 h; (iv) $CH_2(COOC_2H_5)_2$, 200 °C, 2 h; (v) $RCH(COOC_2H_5)_2$, sod. methoxide, methanol, reflux 18 h; (vi) 2-acetylbutyrolactone, bromobenzene, reflux 6 h; (vii) ethyl cyclopentanone-2-carboxylate, 200 °C, 2 h; (viii) 2-acetyl-1-tetralone, gl. acetic acid, reflux 3 h.

as percentage growth inhibition (GI%) caused by the test compounds. The obtained results showed that six compounds, namely; **2**, **3**, **6**, **7**, **13** and **14**, passed successfully this primary anticancer assay and were consequently carried over to the five-dose screen against a panel of about 60 different tumor cell lines.

### 2.2.2. In vitro full panel (five-dose) 60-cell line assay

About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines, were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log concentration-% growth inhibition curves. Three response parameters ($GI_{50}$, TGI, and $LC_{50}$) were calculated for each cell line. The $GI_{50}$ value (growth inhibitory activity) corresponds to the concentration of the compounds causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition and the $LC_{50}$ value (cytotoxic activity) is the concentration of the compounds causing net 50% loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default $GI_{50}$, TGI, or $LC_{50}$ values of all cell lines in the subpanel or the full panel, respectively.




**Scheme 2.** Reagents and reaction conditions: (i) ethoxymethylenemalononitril, abs ethanol, potassium carbonate, reflux 6 h; (ii) ethyl ethoxymethylenecyanoacetate, abs ethanol, potassium carbonate, reflux 8 h; (iii) diethyl ethoxymethylenemalonate, acetonitrile, potassium carbonate, reflux 12 h.

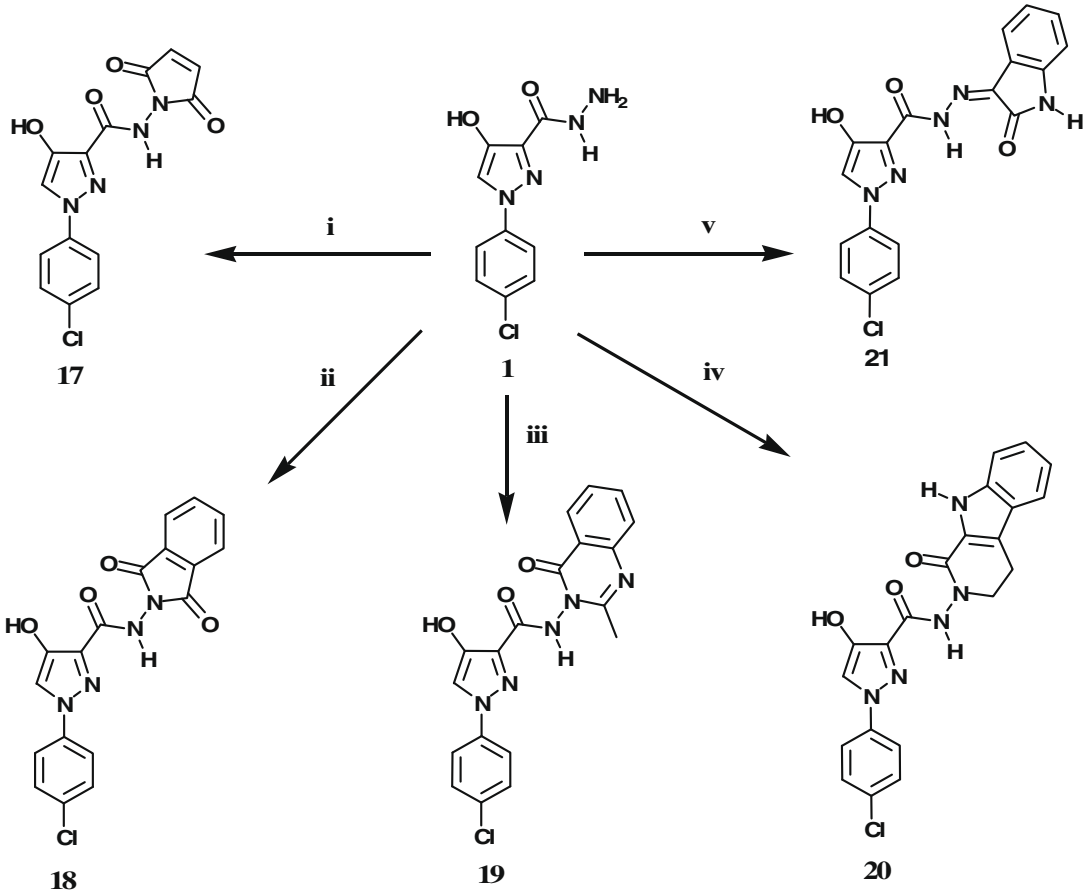
In the present study, the active six compounds; **2**, **3**, **6**, **7**, **13** and **14**, exhibited potential antitumor activities against most of the tested subpanel tumor cell lines ($GI_{50}$, TGI and $LC_{50}$ values <100 μM). These compounds showed a distinctive pattern of sensitivity against some individual cell lines (Table 1), as well as a broad spectrum of antitumor activity (Tables 2 and 3).

Concerning the sensitivity against some individual cell lines (Table 1), compound **2** displayed a distinguished sensitivity profile towards leukemia cell lines with $GI_{50}$ range of 0.42–1.87 μM, in addition to a remarkably high activity against non-small cell lung cancer NCI-H460 and CNS SF-295 cell lines ($GI_{50}$ 0.37 and 0.23 μM, respectively). The analog **3** showed appreciable growth inhibitory activity towards most of the tested subpanel tumor cell lines with $GI_{50}$ range of 1.27–18.5 μM. Furthermore, compound **6** exhibited potential activity toward the renal RXF 393 cell line ($GI_{50}$ value of 0.03 μM). Whereas, compound **7** was able to inhibit the growth of about 50 out of the 60 tested cell lines at $GI_{50}$ value <1 μM, with special high activity against the CNS SF-295 and renal A498 cell lines ($GI_{50}$ 0.15 and 0.11 μM, respectively). On the other hand, compound **13** showed the ever highest growth inhibitory potential against all the tested tumor cell lines, with an outstanding sensitivity profile towards about 47 different cancer cell lines with $GI_{50}$ values lying in the nanomolar concentration range ($GI_{50}$ range 0.02–0.09 μM), in addition to the potential activity against the other cell lines with $GI_{50}$ range of 0.12–5.54 μM. Additionally, compound **14** was able to display a remarkable activity against all the tested tumor cell lines, with $GI_{50}$ value <1 μM.

With regard to the broad spectrum antitumor activity, the results revealed that all of the six active compounds; **2**, **3**, **6**, **7**, **13** and **14**; revealed effective growth inhibition $GI_{50}$ (MG-MID) values of 1.55, 5.75, 2.65, 0.67, 0.08 and 0.36 μM, respectively, beside a cytostatic activity TGI (MG-MID) values of 63.1, 67.6, 77.0, 53.8, 30.9 and 8.78 μM, respectively (Tables 2 and 3). In addition, with the exception of compounds **2** and **3**, compounds **6**, **7**, **13** and **14**; exhibited mild cytotoxic efficacy with $LC_{50}$ (MG-MID) values of 97.7, 93.3, 93.3 and 69.3 μM, respectively (Table 3).

Further interpretation of the obtained data revealed that, compounds **13** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.08, 30.9 and 93.3 μM, respectively) and **14** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.36, 8.78 and 69.3 μM, respectively) proved to be the most active members in this study with a significantly potential activity against all the tested subpanel tumor cell lines at the nanomolar concentration level. Moreover, both compounds revealed distinctive effectiveness on the leukemia subpanel at both the $GI_{50}$ (0.042 and 0.27 μM, respectively) and the TGI levels (10.2 and 3.23 μM, respectively) (Tables 2 and 3). Additionally, compound **14** was able to show a broad spectrum of cytostatic and cytotoxic potentials (TGI and $LC_{50}$ MG-MID ranges of 3.23–23.2 and 44.3–88.5 μM, respectively) against most of the tested subpanel tumor cell lines (Table 3). Compound **7** displayed a remarkable growth inhibitory and cytostatic effects ($GI_{50}$ and TGI MG-MID values 0.67 and 53.8 μM, respectively) when compared with **13** and **14**. On the other hand, compounds **2** ($GI_{50}$ and TGI MG-MID values 1.55 and 63.1 μM, respectively) and **6** ($GI_{50}$ and TGI MG-MID values 2.65 and 77.0 μM, respectively) showed an observable antitumor activity towards most of the tested tumor cell lines with particular high sensitivity towards the leukemia subpanel ($GI_{50}$ and TGI MG-MID values 1.03, 1.76 and 52.9, 80.7 μM, respectively). Finally, compound **3** was found to be relatively the least effective antitumor agent in the present investigation with $GI_{50}$ and TGI (MG-MID) values of 5.75 and 67.6 μM, respectively; and without any cytotoxic effect (Tables 2 and 3).

The ratio obtained by dividing the full panel MG-MID (μM) of the compounds by their individual subpanel MG-MID (μM) is con-




**Scheme 3.** Reagents and reaction conditions: (i) maleic anhydride, anhydrous sodium acetate, gl. acetic acid, reflux 10 h; (ii) phthalic anhydride, xylene, reflux 12 h; (iii) acetanthranil, gl. acetic acid, reflux 14 h; (iv) 3,4-dihydropyrano[3,4-*b*]indol-1(9*H*)-one, acetic acid, reflux 18 h; (v) isatin, acetic acid, reflux 2 h.

sidered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than six indicate high selectivity towards the corresponding cell line, while compounds not meeting either of these criteria are rated non-selective.[39] In this context, the active compounds in the present study were found to be non-selective with broad spectrum antitumor activity against the nine tumor subpanels tested with selectivity ratios ranging between 0.3–2.0 and 0.14–1.2 at the $GI_{50}$ and TGI levels, respectively, except compounds **13** and **14** which showed moderate selectivity towards the leukemia subpanel at the TGI level (selectivity ratios 3.0 and 3.5, respectively).

Structurally, the active analogs **2**, **3**, **6**, **7**, **13** and **14** are bipyrazolyl derivatives linked together through a carbonyl bridge, comprising essentially the 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazol-3-carbonyl counterpart, which was found to be an essential pharmacophore for potential anticancer activity,[18,19] together with other functionalities that could assist in H-bond formation. In this view, the pyrazol-5-one derivative **2** showed remarkable growth inhibitory and cytostatic activities ($GI_{50}$ and TGI MG-MID values 1.55 and 63.1 μM, respectively), while it lacked cytotoxic efficacy. Replacement of the carbonyl functionality at position 5 with a methyl group (**3**; R = $CH_3$), resulted in about threefold reduction of the overall growth inhibitory activity ($GI_{50}$ MG-MID 5.75 μM), whereas its cytostatic and cytotoxic activities were almost not affected. Enlargement of the size of the substituent by introducing an aromatic ring as in compound **4** (R = $C_6H_5$), led to complete abolishment of the activity. Furthermore, increasing compounds' lipophilicity by introducing a trifluromethyl moiety together with changing the nature of substituent at position 3 led to a significant change in the antitumor activity. In this view, when compared with the structure analog **3**, compound **6** (R = 2-furyl) showed about twofold improvement in the growth inhibitory activity ($GI_{50}$ MG-MID 2.65 vs 5.75 μM), while its cytostatic effect was slightly reduced (TGI MG-MID 77.0 vs 67.6 μM), and a weak cytotoxic activity was developed ($LC_{50}$ MG-MID 97.7 μM). On the other hand, introduction of another carbonyl group as in the pyrazolidin-3,5-dione **7** (as a possible H-bond forming center) in place of the methyl group in **2**, resulted in a noticeable enhancement (twofold) in the overall growth inhibition activity ($GI_{50}$ MG-MID 0.67 vs 1.55 μM) and 2–3-fold improvement in the individual subpanel tumor cell lines (Table 2), meanwhile, its cytostatic activity was slightly enhanced (TGI MG-MID 53.8 vs 63.1 μM). However, the same compound showed about fivefold improvement in the cytostatic activity against the leukemia subpanel (TGI MG-MID 10.8 vs 52.9 μM). Here, it should be pointed out that, substituting the second pyrazole moiety with two adjacent highly active functionalities (cyano and amino) led to a potentially active analog **14** which showed a distinctive broad spectrum of antitumor activities at the $GI_{50}$, TGI and $LC_{50}$ MG-MID levels. This compound showed a significant growth inhibitory potential against all the tested subpanel tumor cell lines falling in the sub-micromolar concentration level with ($GI_{50}$ MG-MID range 0.27–0.61 μM), in addition to reliable cytostatic (TGI MG-MID range 3.23–19.6 μM) and cytotoxic ($LC_{50}$ MG-MID range 44.3–88.5 μM) activities. Replacement of the cyano group in compound **14** with a ethoxycarbonyl functionality as in the structure analog **15**, led to a complete abolishment of activity.

Finally, an outstanding profile of antitumor activity was obtained when the substitution pattern was extended to involve

**Table 1**
Growth inhibitory concentrations ($GI_{50}$, μM) of compounds **2**, **3**, **6**, **7**, **13** and **14**.[a]

| Cell lines | 2 | 3 | 6 | 7 | 13 | 14 |
|---|---|---|---|---|---|---|
| *Leukemia* | | | | | | |
| *CCRF-CEM* | 0.42 | 3.66 | 0.69 | 0.42 | 0.057 | 0.28 |
| *HL-60 (TB)* | 1.17 | 3.96 | 2.42 | 0.77 | 0.051 | 0.33 |
| K-562 | 0.65 | NA[b] | 1.72 | 0.29 | 0.030 | 0.25 |
| MOLT-4 | 1.87 | NT[c] | 2.55 | 0.41 | 0.043 | 0.29 |
| RPMI-8226 | 1.66 | 3.34 | 1.42 | 0.38 | 0.038 | 0.21 |
| SR | 0.42 | NA | NT | 0.26 | 0.032 | 0.28 |
| *Non-small cell Lung cancer* | | | | | | |
| A549/ATCC | 0.82 | 3.72 | 7.23 | 0.36 | 0.034 | 0.20 |
| EKVX | 2.21 | 4.87 | 1.99 | 0.85 | 0.071 | 0.38 |
| HOP-62 | 1.33 | 5.21 | 1.98 | 0.51 | 0.044 | 0.34 |
| HOP-92 | 3.67 | 4.52 | 4.90 | 0.50 | 0.052 | 0.40 |
| NCI-H226 | 1.40 | 4.66 | 2.71 | 0.73 | 0.045 | 0.37 |
| NCI-H23 | 2.90 | 3.31 | 5.59 | 0.49 | 0.041 | 0.37 |
| NCI-H322 | 0.85 | 6.17 | 0.83 | 0.53 | 0.054 | 0.44 |
| NCI-H460 | 0.37 | 2.38 | 0.78 | 0.31 | 0.028 | 0.19 |
| NCI-H522 | 2.84 | 7.37 | 2.67 | 0.30 | 0.055 | 0.21 |
| *Colon cancer* | | | | | | |
| COLO 205 | 4.81 | 18.5 | 2.97 | 0.94 | 0.291 | 0.91 |
| HCC-2998 | 3.67 | 15.1 | 10.5 | 4.74 | 0.502 | 0.65 |
| HCT-116 | 0.68 | 3.42 | 2.00 | 0.34 | 0.034 | 0.26 |
| HCT-15 | 0.68 | 3.53 | 1.44 | 0.49 | 0.052 | 0.34 |
| HT29 | 6.16 | 3.29 | 4.56 | 0.58 | 0.241 | 1.19 |
| KM12 | 2.27 | 5.07 | 5.88 | 1.16 | 0.070 | 0.38 |
| SW-620 | 3.41 | 8.02 | 4.75 | 2.32 | 0.360 | 0.52 |
| *CNS Cancer* | | | | | | |
| SF-268 | 0.49 | 4.13 | 3.63 | 0.51 | 0.048 | 0.46 |
| SF-295 | 0.23 | 1.81 | 0.48 | 0.15 | 0.020 | 0.16 |
| SF-539 | 1.70 | 3.05 | 2.81 | 0.37 | 0.041 | 0.32 |
| SNB-19 | 3.16 | 3.60 | 4.37 | 0.51 | 0.045 | 0.45 |
| SNB-75 | 0.53 | 3.52 | 0.82 | 0.24 | 0.029 | 0.25 |
| U251 | 0.41 | 2.68 | 5.86 | 0.18 | 0.031 | 0.29 |
| *Melanoma* | | | | | | |
| LOX IMVI | 1.55 | 3.94 | 2.37 | 1.37 | 0.075 | 0.44 |
| M14 | 1.78 | 4.69 | 4.28 | 0.71 | 0.096 | 0.48 |
| MDA-MB-435 | NT | NT | 5.82 | 0.38 | 0.046 | 0.33 |
| SK-MEL-2 | NT | NT | 3.82 | NA | 5.544 | 0.91 |
| SK-MEL-28 | 3.06 | 6.28 | 9.22 | 5.26 | 0.092 | 0.63 |
| SK-MEL-5 | 1.70 | 6.76 | 2.49 | 1.92 | 0.370 | 0.48 |
| UACC-257 | 1.06 | 16.6 | 2.67 | 1.86 | 0.210 | 0.56 |
| UACC-62 | 1.80 | 3.46 | 2.16 | 0.39 | 0.051 | 0.30 |
| *Ovarian cancer* | | | | | | |
| IGROV1 | 0.51 | 3.16 | 0.92 | 0.38 | 0.043 | 0.36 |
| OVCAR-3 | 1.21 | 1.27 | 1.72 | 0.93 | 0.099 | 0.55 |
| OVCAR-4 | 2.40 | 9.19 | 2.43 | 1.52 | 0.264 | 0.46 |
| OVCAR-5 | 3.32 | 4.98 | 2.60 | 8.08 | 0.072 | 0.64 |
| OVCAR-8 | 1.49 | 5.96 | 1.88 | 0.64 | 0.053 | 0.48 |
| SK-OV-3 | 1.53 | 7.04 | 3.55 | 1.92 | 0.160 | 0.52 |
| *Renal cancer* | | | | | | |
| 786–0 | 6.45 | 9.64 | 6.60 | 3.77 | 0.319 | 0.66 |
| A498 | 0.45 | 2.90 | 8.71 | 0.11 | 0.024 | 0.17 |
| ACHN | 0.88 | 3.77 | 2.53 | 0.58 | 0.048 | 0.32 |
| CAKI-1 | 1.06 | 4.23 | 2.43 | 0.65 | 0.127 | 0.28 |
| RXF 393 | 15.4 | 4.58 | 0.03 | 0.31 | 0.047 | 0.51 |
| SN12C | 1.42 | 3.37 | 5.18 | 0.50 | 0.041 | 0.35 |
| TK-10 | 0.79 | 4.71 | 1.45 | 0.35 | 0.033 | 0.28 |
| UO-31 | 0.67 | 3.01 | 3.84 | 0.55 | 0.099 | 0.30 |
| *Prostate cancer* | | | | | | |
| PC-3 | NT | 7.05 | 26.3 | 0.36 | 0.037 | 0.33 |
| DU-145 | 5.22 | 6.23 | 10.5 | 0.98 | 0.050 | 0.57 |
| *Breast cancer* | | | | | | |
| MCF7 | 3.61 | 4.52 | 3.51 | 1.56 | 0.121 | 0.36 |
| NCI/ADR-RES | 1.89 | 4.42 | 1.60 | 0.94 | 0.046 | 0.37 |
| HS 578T | 0.28 | 3.21 | 0.64 | 0.19 | 0.021 | 0.19 |
| MDA-MB-435 | 1.20 | 3.94 | 1.97 | 0.33 | 0.037 | 0.29 |
| BT-549 | 0.65 | 2.19 | 1.14 | 0.37 | 0.029 | 0.29 |
| T-47D | 2.66 | 3.66 | 4.16 | 0.45 | 0.059 | 0.30 |
| MDA-MB-468 | 4.57 | 8.90 | 4.66 | 0.72 | 0.262 | 0.95 |

[a]Data obtained from NCI's in vitro disease-oriented human tumor cell screen.
[b] NA: Not active ($GI_{50}$ value >100 μM).
[c] NT: Not tested.

the incorporation of a pyrazole moiety in a tricyclic ring system as in the naphthopyrazole **13** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.08, 30.9 and 93.3 μM, respectively). This structure modification resulted in a significant potentiation in the growth inhibitory activity against all the tested subpanel tumor cell lines with $GI_{50}$ MG-MID values lying at the nanomolar concentration level (0.04–0.22 μM), together with a broad spectrum of cytostatic and cytotoxic potentials. This finding substantiates the idea behind the possible role of different linear and semi-linear fused di- and tricyclic ring systems in anticancer activity as DNA intercaletors and kinase inhibitors.[33–35]

## 3. Conclusion

In conclusion, the aim of the present investigation was to synthesize novel 4-hydroxy-1*H*-pyrazole lead compounds possessing potential antitumor activity, as an extension to such previously-reported template of compounds. Such objective has been achieved by the synthesis of two novel series of 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazoles linked to either substituted 1*H*-pyrazole counterparts through a carbonyl bridge, or to some biologically-active nitrogenous heterocycles by an amide linker for synergistic purpose. The most active six compounds **2**, **3**, **6**, **7**, **13** and **14** revealed a significant broad spectrum of antitumor potential against most of the tested subpanel tumor cell lines at the $GI_{50}$ and TGI levels, together with a mild cytotoxic ($LC_{50}$) activity. The pyrazolinedione derivative **7** displayed a remarkable growth inhibition and cytostatic effects ($GI_{50}$ and TGI MG-MID values 0.67 and 53.8 μM, respectively) together with a weak cytotoxic effect. The tricyclic naphthopyrazole **13** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.08, 30.9 and 93.3 μM) and the aminocyanopyrazole **14** ($GI_{50}$, TGI, and $LC_{50}$ MG-MID values 0.36, 8.78 and 69.3 μM, respectively) could be classified as the most active antitumor members identified in this study as evidenced by their significantly potential growth inhibitory activity at the nanomolar concentration level and remarkable cytostatic and cytotoxic activities, regardless to the subpanel being tested, with special high selectivity profile against some individual cell lines.

It is worth-mentioning that, compounds **7**, **13** and **14** were further selected by the NCI's biological committee for subsequent confirmatory screening procedures in order to assess their exact antitumor potentialities. These tests include the repetition of the testing in the primary screen, referring to the Biological Evaluation Committee and a final review by the Biological Evaluation Committee. Interestingly, those three potential compounds have passed successfully all the above-mentioned in vitro stages and are required as candidates for the next in vivo hollow fiber assay.[40]

All these favorable features make such type of 4-hydroxypyrazole derivatives the appropriate candidates for further testing and derivatization in the hope of finding more selective and active anticancer compounds in the nano- or even sub-nanomolar concentration levels.

## 4. Experimental

### 4.1. Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin–Elmer 297 infrared spectrophotometer using the NaCl plate technique. The $^1$H NMR spectra were recorded on a Varian EM 360 spectrometer using tetramethylsilane as the internal standard and DMSO-$d_6$ as the solvent (Chemical shifts in ($\delta$, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Elemen-

**Table 2**
Median growth inhibitory concentrations ($GI_{50}$, μM) of in vitro subpanel tumor cell lines[a]

| Compd no. | Subpanel tumor cell lines[b] | | | | | | | | | MG-MID[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | |
| **2** | 1.03 | 1.82 | 3.09 | 1.09 | 1.82 | 1.74 | 1.67 | 5.22 | 2.02 | 1.55 |
| **3** | 3.65 | 4.69 | 8.13 | 3.13 | 4.95 | 5.27 | 4.52 | 6.64 | 4.96 | 5.75 |
| **6** | 1.76 | 3.19 | 4.59 | 2.99 | 4.14 | 2.18 | 3.85 | 18.4 | 3.07 | 2.65 |
| **7** | 0.42 | 0.51 | 1.51 | 0.33 | 1.60 | 1.08 | 0.85 | 0.67 | 0.82 | 0.67 |
| **13** | 0.042 | 0.047 | 0.221 | 0.036 | 0.140 | 0.115 | 0.092 | 0.044 | 0.082 | 0.08 |
| **14** | 0.27 | 0.32 | 0.61 | 0.32 | 0.46 | 0.50 | 0.36 | 0.45 | 0.43 | 0.36 |

[a] Median values calculated according to the data obtained from NCI's in vitro disease-oriented human tumor cell screen.
[b] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
[c] $GI_{50}$ (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent.

**Table 3**
Median total growth inhibitory concentrations (TGI, μM) and lethal concentration 50 ($LC_{50}$, μM) of in vitro subpanel tumor cell lines[a]

| Compd no. | Subpanel tumor cell lines[b] | | | | | | | | | MG-MID[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | |
| **2** | 52.9 | 78.2 | 95.4 | 79.6 | 70.5 (93.6)[e] | 83.8 | 77.0 | —[d] | 90.1 | 63.1 |
| **3** | 74.3 | 81.7 | 85.1 | 69.2 | 71.4 (92.8) | 87.8 | 71.3 | — | 90.7 | 67.6 |
| **6** | 80.7 | 78.9 | 88.5 | 90.5 | 63.7 (89.4) | 94.5 | 75.6 | — | — | 77.0 (97.7) |
| **7** | 10.8 | 80.3 | 86.2 | 87.9 | 50.1 (85.2) | 84.0 | 71.8 | — | 79.6 | 53.8 (93.3) |
| **13** | 10.2 (90.7) | 68.1 | 86.3 | 69.1 | 52.1 (86.4) | 83.6 | 67.7 | — | 91.7 | 30.9 (93.3) |
| **14** | 3.23 (44.3) | 10.8 (69.4) | 12.6 (64.3) | 23.1 (74.7) | 11.9 (65.0) | 19.6 (88.5) | 8.99 (75.0) | 13.4 | 14.4 (64.3) | 8.78 (69.3) |

[a] Median values calculated according to the data obtained from NCI's in vitro disease-oriented human tumor cell screen.
[b] For subpanel tumor cell lines, see footnote (b) of Table 3.
[c] TGI (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent.
[d] TGI (MG-MID) value >100 μM.
[e] $LC_{50}$ (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent.

tal analyses were performed at the Microanalytical Unit, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia, and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at $\lambda$ 254. The synthesis of the starting 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxylic acid hydrazide **1**,[36] acetanthranil[37] and 3,4-dihydropyrano[3,4-*b*]indol-1(9*H*)-one,[38] was performed according to reported literature procedures.

#### 4.1.1. 3-(4,5-Dihydro-3-methyl-5-oxo-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (2)

Ethyl acetoacetate (0.39 g, 3 mmol) was added to a solution of the acid hydrazide **1** (0.5 g, 2 mmol) in absolute ethanol (15 mL), and the reaction mixture was heated under reflux for 10 h. Solvent was removed under reduced pressure and the remaining residue was recrystallized from aqueous ethanol. Yield: 70%, mp: 284–86 °C, IR ($cm^{-1}$): 3245–2980 (OH), 1740, 1675 (C=O); $^1$H NMR ($\delta$, ppm): 2.09 (s, 3H, C*H*$_3$), 4.24 (s, 2H, C*H*$_2$), 7.38–7.71 (m, 4H, Ar-*H*), 8.23 (s, 1H, pyrazole-C$_5$-*H*), 9.12 (s, 1H, O*H*). Anal. Calcd for $C_{14}H_{11}ClN_4O_3$ (318.72): C, 52.76; H, 3.48; N, 17.58. Found: C, 52.41; H, 3.56; N, 17.39.

#### 4.1.2. General method for the synthesis of 3-(5-methyl-3-substituted-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazoles (3,4) and 3-(3-substituted-5-trifluoromethyl-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazoles (5,6)

To a solution of **1** (0.5 g, 2 mmol) in glacial acetic acid (15 mL), was added the appropriate diketone (2 mmol). The reaction mixture was heated under reflux for 8–12 h, then allowed to cool to room temperature. The solid product thus formed was filtered, thoroughly washed with cold ethanol, dried and recrystallized from ethanol.

**4.1.2.1. 3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (3).** (Using pentane-2,4-dione) Yield: 67%, mp: 260–61 °C, IR ($cm^{-1}$): 3280–3050 (OH), 1670 (C=O); $^1$H NMR ($\delta$, ppm): 2.19 (s, 3H, C*H*$_3$), 2.38 (s, 3H, C*H*$_3$), 6.21 (s, 1H, pyrazole-C$_4$-*H*), 7.43–7.78 (m, 4H, Ar-*H*), 8.27 (s, 1H, pyrazole-C$_5$-*H*), 9.09 (s, 1H, O*H*). Anal. Calcd for $C_{15}H_{13}ClN_4O_2$ (316.74): C, 56.88; H, 4.14; N, 17.69. Found: C, 56.65; H, 4.47; N, 17.31.

**4.1.2.2. 3-(5-Methyl-3-phenyl-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (4).** (Using 1-phenylbutane-1,3-dione) Yield: 38%, mp: 382–84 °C, IR ($cm^{-1}$): 3475–3120 (OH), 1665 (C=O); $^1$H NMR ($\delta$, ppm): 2.49 (s, 3H, C*H*$_3$), 6.30 (s, 1H, pyrazole-C$_4$-*H*), 7.31–7.89 (m, 9H, Ar-*H*), 8.31 (s, 1H, pyrazole-C$_5$-*H*), 9.15 (s, 1H, O*H*). Anal. Calcd for $C_{20}H_{15}ClN_4O_2$ (378.81): C, 63.41; H, 3.99; N, 14.79. Found: C, 63.61; H, 3.78; N, 14.59.

**4.1.2.3. 3-(3-Methyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (5).** (Using 1,1,1-trifluoropentane-2,4-dione) Yield: 63%, mp: 229–31 °C, IR ($cm^{-1}$): 3380–2975 (OH), 1660 (C=O); $^1$H NMR ($\delta$, ppm): 2.32 (s, 3H, C*H*$_3$), 7.19–7.77 (m, 5H, 4 Ar-*H* and pyrazole-C$_4$-*H*), 8.33 (s, 1H, pyrazole-C$_5$-*H*), 9.17 (s, 1H, O*H*). Anal. Calcd for $C_{15}H_{10}ClF_3N_4O_2$ (370.71): C, 48.60; H, 2.72; N, 15.11. Found: C, 48.92; H, 2.48; N, 15.27.

**4.1.2.4. 3-(3(2-Furyl)-5-trifluoromethyl-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (6).** (Using 1-(2-furyl)butane-1,3-dione) Yield: 54%, mp: 280–82 °C, IR ($cm^{-1}$): 3240–3120 (OH), 1665 (C=O); $^1$H NMR ($\delta$, ppm): 7.09–8.27 (m, 8H, 4

Ar-*H*, 3 furyl-*H* and pyrazole-$C_4$-*H*), 8.37 (s, 1H, pyrazole-$C_5$-*H*), 9.16 (s, 1H, O*H*). Anal. Calcd for $C_{18}H_{10}ClF_3N_4O_3$ (422.75): C, 51.14; H, 2.38; N, 13.25. Found: C, 50.89; H, 2.56; N, 13.38.

#### 4.1.3. 3-(3,5-Dioxopyrazolidine-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (7)

A mixture of the starting compound **1** (0.5 g, 2 mmol) and diethyl malonate (0.46 g, 4 mmol) was heated at 200 °C in an oil bath for 2 h. After being cooled to room temperature, the solidified product was treated with cold diethyl ether, filtered, washed with diethyl ether, dried and recrystallized from aqueous ethanol. Yield: 46%, mp: 282–84 °C, IR ($cm^{-1}$): 3370–2755 (OH, NH), 1720, 1700, 1670 (C=O); $^1$H NMR ($\delta$, ppm): 3.51 (s, 2H, $CH_2$), 7.41–7.79 (m, 4H, Ar-*H*), 8.25 (s, 1H, pyrazole-$C_5$-*H*), 9.21 (s, 1H, O*H*), 10.35 (s, 1H, N*H*). Anal. Calcd for $C_{13}H_9ClN_4O_4$ (320.69): C, 48.69; H, 2.83; N, 17.47. Found: C, 48.81; H, 2.64; N, 17.29.

#### 4.1.4. General method for the synthesis of 3-(3,5-dioxo-4-substituted pyrazolidine-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazoles (8–10)

A mixture of **1** (0.5 g, 2 mmol) and the appropriate 2-substituted diethyl malonate (4 mmol) and sodium methoxide (0.22 g, 4 mmol) was refluxed in methanol (20 mL) for 18 h. Excess solvent was removed under reduced pressure, the remaining residue was dissolved in water (20 mL) and extracted with chloroform (2 × 15 mL) to get rid of any unreacted esters. The aqueous solution was acidified with 2 N hydrochloric acid to pH 3–4 to give a creamy white precipitate which was filtered, washed with water, dried and recrystallized from ethanol.

**4.1.4.1. 3-(4-Ethyl-3,5-dioxopyrazolidine-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (8).** (Using diethyl 2-ethylmalonate) Yield: 27%, mp: 242–44 °C, IR ($cm^{-1}$): 3420–2810 (OH, NH), 1725, 1700, 1675 (C=O); $^1$H NMR ($\delta$, ppm): 1.09 (t, 3H, $CH_3$), 2.11 (q, 2H, $CH_2$), 3.38–3.44 (m, 1H, pyrazole-$C_4$-*H*), 7.37–7.81 (m, 4H, Ar-*H*), 8.22 (s, 1H, pyrazole-$C_5$-*H*), 9.24 (s, 1H, O*H*), 10.47 (s, 1H, N*H*). Anal. Calcd for $C_{15}H_{13}ClN_4O_4$ (348.74): C, 51.66; H, 3.76; N, 16.07. Found: C, 51.48; H, 3.88; N, 15.93.

**4.1.4.2. 3-(4-Butyl-3,5-dioxopyrazolidine-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (9).** (Using diethyl 2-butylmalonate) Yield: 32%, mp: 250–52 °C, IR ($cm^{-1}$): 3460–2670 (OH, NH), 1720, 1710, 1665 (C=O); $^1$H NMR ($\delta$, ppm): 1.01–1.52 (m, 9H, *n*-butyl-9*H*), 3.32–3.37 (m, 1H, pyrazole-$C_4$-*H*), 7.41–7.76 (m, 4H, Ar-*H*), 8.23 (s, 1H, pyrazole-$C_5$-*H*), 9.19 (s, 1H, O*H*), 10.38 (s, 1H, N*H*). Anal. Calcd for $C_{17}H_{17}ClN_4O_4$ (376.79): C, 54.19; H, 4.55; N, 14.87. Found: C, 53.94; H, 4.67; N, 14.68.

**4.1.4.3. 3-(3,5-Dioxo-4-phenylpyrazolidine-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (10).** (Using diethyl 2-phenylmalonate) Yield: 41%, mp: 357–59 °C, IR ($cm^{-1}$): 3460–2670 (OH, NH), 1715, 1700, 1670 (C=O); $^1$H NMR ($\delta$, ppm): 4.97 (s, 1H, pyrazole-$C_4$-*H*), 7.29–7.78 (m, 9H, Ar-*H*), 8.21 (s, 1H, pyrazole-$C_5$-*H*), 9.17 (s, 1H, O*H*), 10.45 (s, 1H, N*H*). Anal. Calcd for $C_{19}H_{13}ClN_4O_4$ (396.78): C, 57.51; H, 3.30; N, 14.12. Found: C, 57.64; H, 3.07; N, 14.26.

#### 4.1.5. 3-(1,2-Dihydro-5-(2-hydroxyethyl)-4-methyl-3-oxopyrazol-2-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (11)

A solution of the acid hydrazide **1** (0.5 g, 2 mmol) and 2-acetylbutyrolactone (0.38 g, 3 mmol) in bromobenzene (10 mL), was refluxed for 6 h. After cooling to room temperature, the separated solid product was filtered, washed with cold ethanol, dried and recrystallized from ethanol. Yield: 32%, mp: 179–81 °C, IR ($cm^{-1}$): 3640–3020 (OH, NH), 1690, 1670 (C=O); $^1$H NMR ($\delta$, ppm): 1.94 (s, 3H, $CH_3$), 2.42 (t, 2H, $CH_2$), 3.51 (t, 2H, –$CH_2$–), 6.79 (s, 1H, –$CH_2$–O*H*), 7.41–7.69 (m, 4H, Ar-*H*), 8.19 (s, 1H, pyrazole-$C_5$-*H*), 9.08 (s, 1H, pyrazole-4-O*H*), 10.38 (s, 1H, N*H*). Anal. Calcd for $C_{16}H_{15}ClN_4O_4$ (362.77): C, 52.97; H, 4.17; N, 15.44. Found: C, 52.76; H, 4.35; N, 15.12.

#### 4.1.6. 3-(3-Oxo-1,2,5,6-tetrahydrocyclopenta[*c*]pyrazol-2-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (12)

The acid hydrazide **1** (0.5 g, 2 mmol) and ethyl cyclopentanone-2-carboxylate (0.47 g, 3 mmol) were heated at 200 °C in an oil bath for 2 h. After being cooled to room temperature, the solidified product was treated with cold methanol, filtered, washed with cold methanol, dried and recrystallized from toluene/petroleum ether (60/80). Yield: 65%, mp: 188–90 °C, IR ($cm^{-1}$): 3350–2860 (OH, NH), 1690, 1665 (C=O); $^1$H NMR ($\delta$, ppm): 1.76–1.79 (m, 2H, $CH_2$), 2.70 (t, 2H, $CH_2$), 2.92 (t, 2H, $CH_2$), 7.29–7.61 (m, 4H, Ar-*H*), 8.16 (s, 1H, pyrazole-$C_5$-*H*), 8.99 (s, 1H, O*H*), 9.96 (s, 1H, N*H*). Anal. Calcd for $C_{16}H_{13}ClN_4O_3$ (344.75): C, 55.74; H, 3.80; N, 16.25. Found: C, 55.87; H, 3.55; N, 16.08.

#### 4.1.7. 3-(3-Methyl-4,5-dihydronaphtho[1,2-*c*]pyrazol-2-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (13)

To a solution of **1** (0.5 g, 2 mmol) in glacial acetic acid (10 mL), was added 2-acetyl-1-tetralone (0.38 g, 2 mmol). The reaction mixture was refluxed for 3 h, during which a heavy yellow solid was crystallized out. After cooling to room temperature, the separated product was filtered, washed with cold ethanol, dried and recrystallized from ethanol. Yield: 54%, mp: 363–65 °C, IR ($cm^{-1}$): 3230–2780 (OH), 1670 (C=O); $^1$H NMR ($\delta$, ppm): 2.39 (s, 3H, $CH_3$), 2.62–2.74 (m, 4H, 2 $CH_2$), 7.38–8.09 (m, 9H, 8 Ar-*H* and pyrazole-$C_5$-*H*), 8.87 (s, 1H, O*H*). Anal. Calcd for $C_{22}H_{17}ClN_4O_2$ (404.85): C, 65.27; H, 4.23; N, 13.84. Found: C, 65.04; H, 4.51; N, 13.69.

#### 4.1.8. 3-(5-Amino-4-cyano-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (14)

A mixture of **1** (0.5 g, 2 mmol), ethoxymethylenemalononitrile (0.24 g, 2 mmol) and anhydrous potassium carbonate (0.3 g, 2 mmol) in absolute ethanol (15 mL) was heated under reflux for 6 h. After being cooled to room temperature, the yellowish solid product was separated, suspended in water (10 mL) and acidified with 2 N hydrochloric acid till pH 3–4. The resulting creamy white precipitate was filtered, washed thoroughly with water, dried and recrystallized from ethanol. Yield: 79%, mp: 288–90 °C, IR ($cm^{-1}$): 3690–3050 (OH, NH), 2215 (CN), 1665 (C=O); $^1$H NMR ($\delta$, ppm): 6.73 (s, 2H, N$H_2$), 7.46–7.98 (m, 5H, 4 Ar-*H* and pyrazole-$C_3$-*H*), 8.36 (s, 1H, pyrazole-$C_5$-*H*), 9.12 (s, 1H, O*H*). Anal. Calcd for $C_{14}H_9ClN_6O_2$ (328.71): C, 51.15; H, 2.76; N, 25.57. Found: C, 51.34; H, 2.65; N, 25.34.

#### 4.1.9. 3-(5-Amino-4-ethoxycarbonyl-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (15)

The title compound was prepared as described for compound **14** by refluxing a mixture of **1** (0.5 g, 2 mmol), ethyl ethoxymethylenecyanoacetate (0.35 g, 2 mmol) and anhydrous potassium carbonate (0.3 g, 2 mmol) in absolute ethanol (15 mL) for 8 h. Yield: 82%, mp: 267–69 °C, IR ($cm^{-1}$): 3580–2940 (OH, NH), 1665, 1715 (C=O); $^1$H NMR ($\delta$, ppm): 1.29 (t, 3H, $CH_3$), 3.47 (q, 2H, $CH_2$); 6.61 (s, 2H, N$H_2$), 7.59–8.07 (m, 5H, 4 Ar-*H* and pyrazole-$C_3$-*H*), 8.31 (s, 1H, pyrazole-$C_5$-*H*), 9.09 (s, 1H, O*H*). Anal. Calcd for $C_{16}H_{14}ClN_5O_4$ (375.77): C, 51.14; H, 3.76; N, 18.64. Found: C, 50.93; H, 3.87; N, 18.55.

#### 4.1.10. 3-(4-Ethoxycarbonyl-5-hydroxy-1*H*-pyrazol-1-yl)carbonyl-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole (16)

An equimolar mixture of **1** (0.5 g, 2 mmol), diethyl ethoxymethylenemalonate (0.45 g, 2 mmol) and anhydrous potassium carbonate (0.3 g, 2 mmol) in acetonitrile (15 mL) was refluxed for 12 h. The reaction mixture was concentrated in vacuo, and allowed to attain room temperature. The separated solid product was suspended in water (10 mL) and acidified with 2 N hydrochloric acid till pH 3–4. The resulting creamy white precipitate was filtered, washed thoroughly with water, dried and recrystallized from ethanol. Yield: 91%, mp: 294–96 °C, IR ($cm^{-1}$): 3470–2710 (OH), 1725, 1670 (C=O); $^1$H NMR ($\delta$, ppm): 1.34 (t, 3H, C*H*$_3$), 4.25 (q, 2H, C*H*$_2$); 7.67–8.12 (m, 5H, 4 Ar-*H* and pyrazole-C$_3$-*H*), 8.39 (s, 1H, pyrazole-C$_5$-*H*), 8.92 (s, 1H, O*H*), 10.20 (s, 1H, O*H*). Anal. Calcd for $C_{16}H_{13}ClN_4O_5$ (376.75): C, 51.01; H, 3.48; N, 14.87. Found: C, 50.86; H, 3.63; N, 14.64.

#### 4.1.11. *N*-(2,4-Dioxo-2,5-dihydropyrrol-1-yl)-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxamide (17)

A mixture of the acid hydrazide **1** (0.5 g, 2 mmol), maleic anhydride (0.2 g, 2 mmol) and anhydrous sodium acetate (0.2 g, 2.5 mmol) in glacial acetic acid (20 mL) was heated under reflux for 10 h. The reaction mixture was concentrated in vacuo to half its volume and allowed to cool. The precipitated solid product was filtered, washed with cold ethanol, dried and recrystallized from acetic acid. Yield: 37%, mp: 236–38 °C, IR ($cm^{-1}$): 3490–2820 (OH, NH), 1705, 1680, 1665 (C=O); $^1$H NMR ($\delta$, ppm): 6.44–6.48 (m, 2H, olefinic 2*H*), 7.54–8.01 (m, 5H, 4 Ar-*H* and pyrazole-C$_5$-*H*), 8.45 (s, 1H, N*H*), 9.15 (s, 1H, O*H*). Anal. Calcd for $C_{14}H_9ClN_4O_4$ (332.7): C, 50.54; H, 2.73; N, 16.84. Found: C, 50.38; H, 2.81; N, 16.69.

#### 4.1.12. *N*-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxamide (18)

A mixture of the acid hydrazide **1** (0.5 g, 2 mmol) and phthalic anhydride (0.3 g, 2 mmol) in xylene (10 mL) was heated under reflux for 12 h. After cooling, the obtained solid product was filtered, washed with cold ethanol, and recrystallized from acetic acid containing few drops of water. Yield: 88%, mp: 325–27 °C, IR ($cm^{-1}$): 3370–2730 (OH, NH), 1735, 1700, 1665 (C=O); $^1$H NMR ($\delta$, ppm): 7.33–7.96 (m, 8H, Ar-*H*), 8.43 (s, 1H, pyrazole-C$_5$-*H*), 8.76 (s, 1H, N*H*), 9.32 (s, 1H, O*H*). Anal. Calcd for $C_{18}H_{11}ClN_4O_4$ (382.76): C, 56.48; H, 2.90; N, 14.64. Found: C, 56.71; H, 2.69; N, 14.53.

#### 4.1.13. 1-(4-Chlorophenyl)-4-hydroxy-*N*-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)-1*H*-pyrazole-3-carboxamide (19)

To a solution of the acid hydrazide **1** (0.5 g, 2 mmol) in glacial acetic acid (10 mL) was added acetanthranil (0.32 g, 2 mmol) and the reaction mixture was heated under reflux for 14 h. After being cooled to room temperature, the mixture was poured onto crushed ice and the separated solid product was filtered, thoroughly washed with water and dried. It was recrystallized from dioxan containing few drops of water. Yield: 48%, mp: 229–31 °C, IR ($cm^{-1}$): 3240–2810 (OH, NH), 1690, 1665 (C=O); $^1$H NMR ($\delta$, ppm): 2.51 (s, 3H, C*H*$_3$), 7.41–7.86 (m, 8H, Ar-*H*), 8.41 (s,1H, pyrazole-C$_5$-*H*), 8.85 (s, 1H, N*H*), 9.07 (s, 1H, O*H*). Anal. Calcd for $C_{19}H_{14}ClN_5O_3$ (395.8): C, 57.66; H, 3.57; N, 17.69. Found: C, 57.46; H, 3.66; N, 17.51.

#### 4.1.14. 1-(4-Chlorophenyl)-*N*-(3,4-dihydro-1-oxo-1*H*-pyrido[3,4-b]indol-2(9*H*)-yl)-4-hydroxy-1*H*-pyrazole-3-carboxamide (20)

A mixture of the acid hydrazide **1** (0.5 g, 2 mmol) and 3,4-dihydropyrano[3,4-*b*]indol-1(9*H*)-one (0.37 g, 2 mmol) in glacial acetic acid (20 mL) was heated under reflux for 18 h. working up of the reaction mixture was carried out as described under compound **19**. Yield: 35%, mp: 274–75 °C, IR ($cm^{-1}$): 3450–2920 (OH, NH), 1670, 1645 (C=O); $^1$H NMR ($\delta$, ppm): 3.36 (t, 2H, C*H*$_2$), 3.51 (t, 2H, C*H*$_2$), 7.19–7.79 (m, 8H, Ar-*H*), 8.40 (s, 1H, pyrazole-C$_5$-*H*), 8.91 (s, 2H, amide N*H* and indole-N*H*), 9.10 (s, 1H, O*H*). Anal. Calcd for $C_{21}H_{16}ClN_5O_3$ (421.84): C, 59.79; H, 3.82; N, 16.60. Found: C, 59.63; H, 3.91; N, 16.44.

#### 4.1.15. 1-(4-Chlorophenyl)-4-hydroxy-3-(2-oxoindolin-3-ylidene)hydrazinocarbonyl)-1*H*-pyrazole (21)

A solution of the acid hydrazide **1** (0.5 g, 2 mmol) and isatin (0.3 g, 2 mmol) in glacial acetic acid (10 mL) was heated under reflux for 2 h, during which a deep yellow solid was partially crystallized out. The solid separated upon cooling was filtered, washed with cold ethanol and recrystallized from ethanol. Yield: 86%, mp: 346–48 °C, IR ($cm^{-1}$): 3460–3040 (OH, NH), 1690, 1655 (C=O); $^1$H NMR ($\delta$, ppm): 7.08–7.69 (m, 8H, Ar-*H*), 8.35 (s, 1H, pyrazole-C$_5$-*H*), 9.24 (s, 2H, amide N*H* and indole-N*H*), 9.55 (s, 1H, O*H*). Anal. Calcd for $C_{18}H_{12}ClN_5O_3$ (381.77): C, 56.63; H, 3.17; N, 18.34. Found: C, 56.47; H, 3.06; N, 18.12.

### 4.2. In vitro antitumor activity

#### 4.2.1. Preliminary in vitro one-dose antitumor screening

Out of the newly synthesized compounds, ten derivatives namely; **2**–**7**, **13**–**15** and **18**; were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro anticancer activity. Primary in vitro one-dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda.[29–31] These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48 h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth. Six compounds namely; **2**, **3**, **6**, **7**, **13** and **14**, passed this primary anticancer assay and consequently were carried over to the five-dose screen against a panel of about 60 different tumor cell lines.

#### 4.2.2. Full in vitro five-dose antitumor assay

Compounds **2**, **3**, **6**, **7**, **13** and **14** were subjected to the NCI in vitro disease-oriented human cells screening panel assay to screen their antitumor activities. About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines were utilized. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 mL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% $CO_2$, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs were solubilized in dimethyl sulphoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg $mL^{-1}$ gentamicin. Additional four, 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 ml of these different drug dilutions were added to the appropriate microtiter wells already containing 100 ml of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional

48 h at 37 °C, 5% $CO_2$, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 ml of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulphorhodamine B (SRB) solution (100 ml) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 ml of 80% TCA (final concentration, 16% TCA). Three response parameters ($GI_{50}$, TGI, and $LC_{50}$) were calculated for each cell line.[29–31]

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