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Short Report

# Potential Antileishmanial Activity of 4-*N*-Acylhydrazone Pyrazolo[3,4-*d*]pyridazin-7-ones: Synthesis, *in vitro* Biological Evaluations and Computational Studies

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In this work a series of 28 novel pyrazolo[3,4-*d*]pyridazin-7-ones were synthesized and tested against *Leishmania amazonensis* (strain WHOM/BR/75/JOSEFA) in promastigote and axenic amastigote forms. Five compounds were active against both cellular forms with $IC_{50}$ (inhibitory concentration growth of 50%) values of 20.2, 11.7, 16.2, 29.5 and 40.3 μM for promastigote and 17.4, 25.2, 3.84, 21.8 and 22.7 μM for axenic amastigote. All compounds were studied by the Lipinski rule, cytotoxicity both *in silico* and *in vitro* to fibroblast line (L929) and macrophages (J774A1), and the most active compound showed a selectivity index of 59.9.

**Keywords:** pyrazolo[3,4-*d*]pyridazinone, *N*-acylhydrazone, *Leishmania amazonensis*

## Introduction

Leishmaniasis is a major infection, a neglected disease caused by more than 20 species that belong to family Trypanosomatidae and genus Leishmania. This disease is distributed worldwide and can be found in subtropical and temperate regions in more than 88 countries, mainly affecting poor regions of developing countries. It is estimated that about 12-15 million people are already infected with leishmaniasis; 2 million new cases occur annually and around 350 million people live in risk areas. The parasites may be transferred to humans by bites from 30 species of sand fly and can cause three main clinical types of leishmaniasis: cutaneous, mucocutaneous and visceral.[1-7] The drugs used nowadays in the treatment of leishmaniasis have shown highly toxic effects and parasite resistance, demonstrating the necessity of development of novel antileishmanial compounds.[8-10]

It is known that pyrazoles,[11-14] fused pyrazoles[15-17] and *N*-acylhydrazones[13,18] show antileishmanial activity. Recently, studies by our research group have shown the synthesis,[19] and antileishmanial activity[20] of a series of 1,4,6-trisubstituted pyrazolo[3,4-*d*]pyridazin-7-one-*N*-acylhydrazone-(bi)thiophene hybrids. In continuation of our studies with pyrazolopyridazinone hybrids, a new series was synthesized using a phenyl substituent in the *N*-acylhydrazone moiety, which is a bioisostere of thiophene (Figure 1).

## Results and Discussion

The novel pyrazolo[3,4-*d*]pyridazin-7-ones **5**-**8** were obtained from the β-enamino diketone and hydrazines according to highly regioselective methodology reported by our research group,[19] as shown in Scheme 1, and characterized by $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR), heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) data, high resolution mass spectrometry (HRMS), X-ray diffraction (XRD, CCDC 1576799) and melting point (mp) (see Supplementary Information for full details).

All new compounds synthesized (**3b**, **4b**, **5e**-**j**, **6e**-**l**, **7e**-**j** and **8e**-**j**) were evaluated against promastigote and axenic amastigote forms of *L. amazonensis* and the inhibitory

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concentration growth of 50% ($IC_{50}$) was determined for each compound. Results of $IC_{50} > 100$ μM were considered as inactive. As observed for the compounds **3a**,**c**,**d** and **4a**,**c**,**d**,[20] the ester (**3b**) and hydrazide (**4b**) derivatives were also inactive against *L. amazonensis*, with values of $IC_{50}$ higher than 100 μM for both, although the formation of

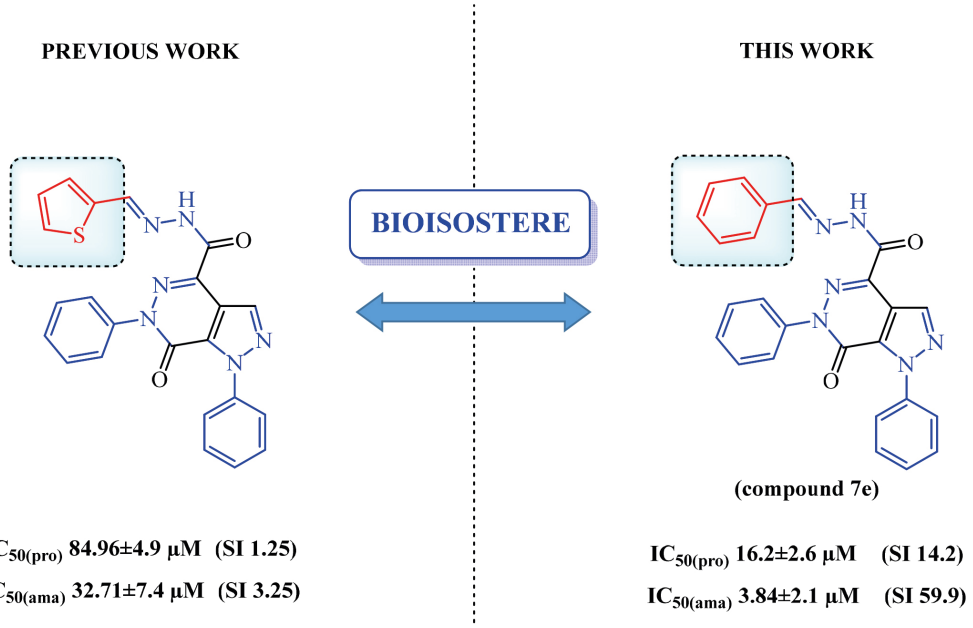
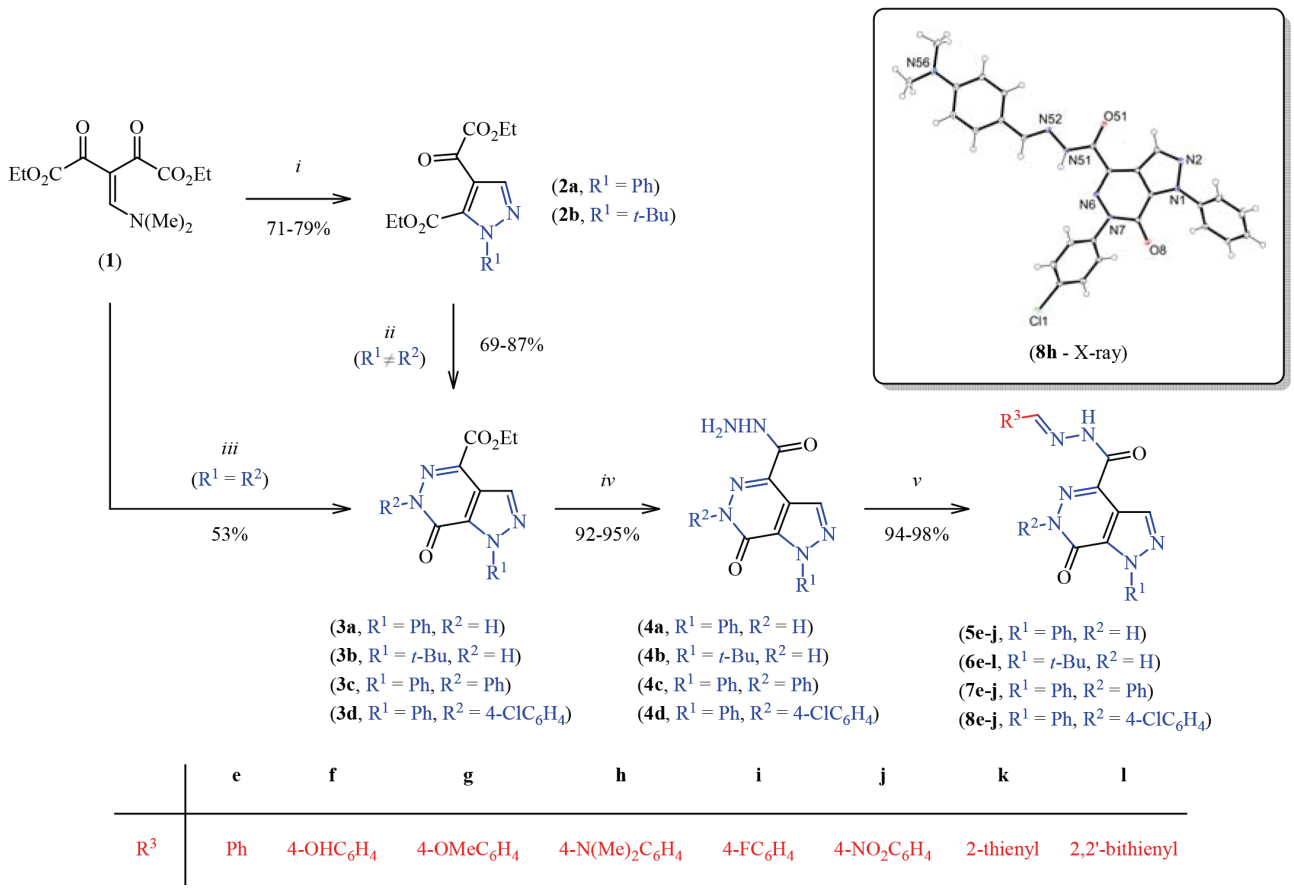



**Figure 1.** Pyrazolopyridazinones synthesized in previous work[20] and in this work.




| | e | f | g | h | i | j | k | l |
|---|---|---|---|---|---|---|---|---|
| $R^3$ | Ph | 4-$OHC_6H_4$ | 4-$OMeC_6H_4$ | 4-$N(Me)_2C_6H_4$ | 4-$FC_6H_4$ | 4-$NO_2C_6H_4$ | 2-thienyl | 2,2'-bithienyl |

**Scheme 1.** General methodology for synthesis of new derivatives. (*i*) $R^1NHNH_2$ (0.9 equiv.), EtOH; (*ii*) $R^2NHNH_2$ (1.1 equiv.), AcOH (4.0 equiv.), EtOH; (*iii*) $PhNHNH_2$ (2.2 equiv.), AcOH (4.0 equiv.), EtOH; (*iv*) $NH_2NH_2.H_2O$ (20 equiv.), EtOH:MeCN (1:1); (*v*) aldehyde($R^3$) (1.0 equiv.), $HCl_{(cat.)}$, DMSO.

the phenyl-substituted pyrazolo[3,4-*d*]pyridazin-7-one-*N*-acylhydrazone **5**-**8** led to active compounds **6h**, **6l**, **7e**, **8f**, and **8i** with $IC_{50}$ values of 20.2, 11.7, 16.2, 29.5 and 40.3 μM to promastigote forms and 17.4, 25.2, 3.84, 21.8 and 22.7 μM to axenic amastigote forms, respectively (Table 1).

It is notorious that the *N*-acylhydrazone moiety in 4-position has a great influence on the activity;[20] however, the nature of the substituent in 1 and 6-positions of pyrazolo[3,4-*d*]pyridazinone also has an influence on the antileishmanial results for those substances (Figure 2), where the presence of a phenyl group in all positions (1, 4 and 6) resulted in substance **7e**, which showed the best results of $IC_{50}$: 16.2 and 3.84 μM for promastigotes and amastigotes, respectively. The phenyl substituent can be found in 1-position in two more active compounds **8f** and **8i**, where there is a 4-chlorophenyl substituent in 6-position; nevertheless, this combination led to less active compounds. Compounds **6h** and **6l**, which have not phenyl substituent in 1 or 6-position, were active with $IC_{50}$ of 20.2 and 11.7, and 17.4 and 25.2 μM for promastigotes and amastigotes, respectively. Although these results demonstrated that *N*-acylhydrazone moiety in 4-position and the nature of the substituent in 1 and 6-positions of pyrazolo[3,4-*d*] pyridazinone play an important role in antileishmanial activity, additional studies are necessary to investigate the action mechanism of these class of compounds in antileishmanial activity.

**Table 1.** *In vitro* antiproliferative activity in *L. amazonensis* and cytotoxicity in mammalian cells of new compounds synthesized

| Compound | $IC_{50(pro)}$ [a] / μM | $IC_{50(ama)}$ [b] / μM | $CC_{50(f)}$ [c] / μM | $CC_{50(m)}$ [d] / μM | $SI_{p\text{-}f}$ [e] | $SI_{p\text{-}m}$ [f] | $SI_{a\text{-}f}$ [g] | $SI_{a\text{-}m}$ [h] |
|---|---|---|---|---|---|---|---|---|
| **3b** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **4b** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **5e** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **5f** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **5g** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **5h** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **5i** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **5j** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6e** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6f** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6g** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6h** | 20.2 ± 0.2 | 17.4 ± 2.1 | 228.5 ± 9.1 | 234.0 ± 5.7 | 11.3 | 11.6 | 13.1 | 13.4 |
| **6i** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6j** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6k** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **6l** | 11.7 ± 1.1 | 25.2 ± 2.7 | 301.7 ± 7.4 | 298.5 ± 8.8 | 25.8 | 25.5 | 12.0 | 11.8 |
| **7e** | 16.2 ± 2.6 | 3.84 ± 2.1 | 145.54 ± 14.45 | 230.15 ± 10.8 | 9.0 | 14.2 | 37.9 | 59.9 |
| **7f** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **7g** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **7h** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **7i** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **7j** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **8e** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **8f** | 29.5 ± 3.0 | 21.8 ± 1.4 | 245.1 ± 7.8 | 370.8 ± 9.0 | 8.3 | 12.6 | 11.2 | 17.0 |
| **8g** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **8h** | > 100 | > 100 | – | > 100 | – | – | – | – |
| **8i** | 40.3 ± 4.4 | 22.7 ± 0.9 | 273.2 ± 9.6 | 318.3 ± 3.6 | 6.8 | 7.9 | 12.0 | 14.0 |
| **8j** | > 100 | > 100 | – | > 100 | – | – | – | – |

[a]$IC_{50}$ (inhibitory concentration growth of 50%) values of promastigote form; [b]$IC_{50}$ values of axenic amastigote form; [c]cytotoxic concentration corresponding to 50% inhibition of fibroblast growth; [d]cytotoxic concentration corresponding to 50% inhibition of macrophage growth; [e]selectivity index ($CC_{50(f)}/IC_{50(pro)}$); [f]selectivity index ($CC_{50(m)}/IC_{50(pro)}$); [g]selectivity index ($CC_{50(f)}/IC_{50(ama)}$); [h]selectivity index ($CC_{50(m)}/IC_{50(ama)}$). –: not determined.

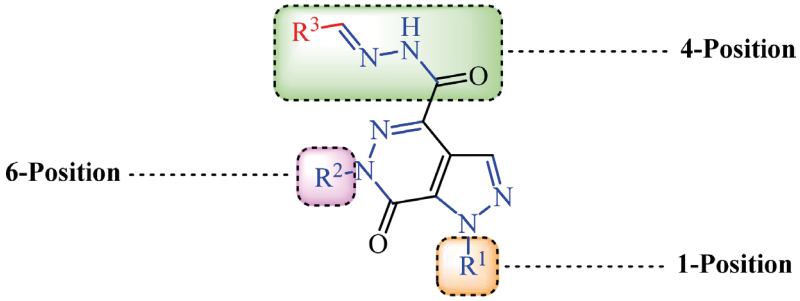



**Figure 2.** Influence of substituent in different positions.

The cytotoxicity of all compounds was investigated in mammalian fibroblast L929 cells and macrophages J774A1 through the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method.[21] For the active compound the range of cytotoxicity was up to 1000 μM. The most active compound **7e** was also the most selective against both forms of *L. amazonensis*, with selectivity index (SI) values of 37.9 and 59.9 to promastigote and amastigote forms, respectively. All the active compounds showed SI values higher than 10 for axenic amastigote forms, and all inactive compounds showed values of cytotoxic concentration corresponding to 50% inhibition ($CC_{50}$) > 100 μM.

In addition, all compounds were evaluated considering the Lipinski rules,[22] aiming to determine the theoretical oral bioavailability using Molinspiration online software.[23] Some descriptors, such as nRot (number of rotatable bonds) and PSA (polar surface area) were included in the Lipinski rules, in which is desirable that there are 10 or fewer rotatable bonds and a polar surface area equal to or less than 140 Å$^2$ (Table 2). Five compounds, **5j**, **6j**, **7j**, **8h** and **8j** showed one or two violations of Lipinski rules and may present problems in oral bioavailability; however, these did not show antileishmanial activity. All the active derivatives, **6h**, **6l**, **7e**, **8f** and **8i** showed no violations of conditions, being able to participate in the drug development process (Table 2).

The calculated electrostatic potential map (MEP) showed no important differences between the compounds studied. Considering compound **8f**, regions of negative electrostatic potential near the nitrogen atom of the pyrazole ring, the oxygen atoms of the carbonyl moieties located on the lateral chain and the pyridazinone group were observed (Figure 3), and the positive electrostatic potentials were found in the lateral chain in all compounds studied.

For *in silico* toxicological studies, Osiris Property Explorer software[25] was used for calculating the fragment-based toxicity risks, and compared with hydrogen peroxide and meglumine antimoniate (Table 3).

The theoretical toxicity evaluation of the tumorigenic profiles of pyrazolo[3,4-*d*]pyridazin-7-one derivatives **4b**, **6h** and **8h** showed the same tumorigenic risk as $H_2O_2$. The compound **4b** has an hydrazide moiety without substitution, and **6h** and **8h** have a dimethylamino attached to the *para*-position of the benzene ring, which

**Table 2.** Physicochemical descriptors calculated for the set of pyrazolo[3,4-*d*]pyridazinone derivatives

| Compound | Molecular weight / Dalton | nON | nOHNH | miLogP | nRot | PSA / Å$^2$ |
|---|---|---|---|---|---|---|
| **5e** | 358.36 | 8 | 2 | 2.40 | 4 | 105.04 |
| **5f** | 374.36 | 9 | 3 | 1.93 | 4 | 125.27 |
| **5g** | 388.39 | 9 | 2 | 2.46 | 5 | 114.28 |
| **5h** | 401.43 | 9 | 2 | 2.51 | 6 | 108.28 |
| **5i** | 376.35 | 8 | 2 | 2.57 | 4 | 105.04 |
| **5j** | 406.36 | 11 | 2 | 2.36 | 5 | 150.87 |
| **6e** | 338.37 | 8 | 2 | 2.32 | 4 | 105.04 |
| **6f** | 354.37 | 9 | 3 | 1.84 | 4 | 125.27 |
| **6g** | 368.40 | 9 | 2 | 2.37 | 5 | 114.28 |
| **6h** | 381.44 | 9 | 2 | 2.42 | 5 | 140.28 |
| **6i** | 356.36 | 8 | 2 | 2.48 | 4 | 102.04 |
| **6j** | 383.37 | 11 | 2 | 2.27 | 5 | 150.87 |
| **6k** | 346.42 | 8 | 2 | 1.07 | 4 | 98.53 |
| **6l** | 428.53 | 8 | 2 | 2.77 | 5 | 98.53 |
| **7e** | 434.46 | 8 | 1 | 3.75 | 5 | 94.19 |
| **7f** | 450.46 | 9 | 2 | 3.27 | 5 | 114.41 |
| **7g** | 464.49 | 9 | 1 | 3.80 | 6 | 103.42 |
| **7h** | 477.53 | 9 | 1 | 3.45 | 6 | 97.82 |
| **7i** | 452.45 | 8 | 1 | 3.91 | 5 | 94.19 |
| **7j** | 479.46 | 11 | 1 | 3.71 | 6 | 140.01 |
| **8e** | 468.90 | 8 | 1 | 4.42 | 5 | 94.19 |
| **8f** | 484.90 | 9 | 2 | 3.95 | 5 | 114.41 |
| **8g** | 498.93 | 9 | 1 | 4.48 | 6 | 103.42 |
| **8h** | 511.97 | 9 | 1 | 4.53 | 6 | 97.42 |
| **8i** | 486.89 | 8 | 1 | 4.59 | 5 | 94.19 |
| **8j** | 513.90 | 11 | 2 | 4.38 | 6 | 140.31 |

nRot: number of rotatable bonds; PSA: polar surface area.

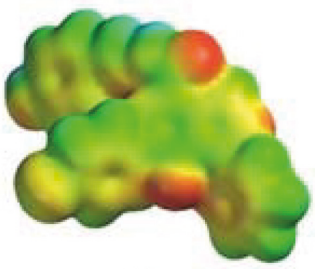



**Figure 3.** 3D visualization of the MEP from compound **8f**, beyond the Van der Waals surface using Spartan for Window 08.[24] Color scheme: blue positive to red negative electrostatic potentials values (–40.000 to 67.000 kcal $mol^{-1}$).

**Table 3.** Theoretical toxicity evaluation values of the active compounds pyrazolo[3,4-*d*]pyridazin-7-one derivatives compared to $H_2O_2$ and meglumine antimoniate

| Compound | Toxicity risk | | | |
|---|---|---|---|---|
| | Mutagenic | Tumorigenic | Irritant | Reproductive |
| **3b** | (+) | (+) | (+) | (+) |
| **4b** | (+++) | (+++) | (+) | (+) |
| **5e** | (+) | (+) | (+) | (+) |
| **5f** | (+) | (+) | (+) | (+) |
| **5g** | (+) | (+) | (+) | (+) |
| **5h** | (+) | (+) | (+) | (+) |
| **5i** | (+) | (+) | (+) | (+) |
| **5j** | (+) | (+) | (+++) | (+) |
| **6e** | (+) | (+) | (+) | (+) |
| **6f** | (+) | (+) | (+) | (+) |
| **6g** | (+) | (+) | (+) | (+) |
| **6h** | (+) | (+++) | (+) | (+) |
| **6i** | (+) | (+) | (+) | (+) |
| **6j** | (+) | (+) | (+) | (+) |
| **6k** | (+) | (+) | (+) | (+) |
| **6l** | (+) | (+) | (+) | (+) |
| **7e** | (+) | (+) | (+) | (+) |
| **7f** | (+) | (+) | (+) | (+) |
| **7g** | (+) | (+) | (+) | (+) |
| **7h** | (+) | (+) | (+) | (+) |
| **7i** | (+) | (+) | (+) | (+) |
| **7j** | (+) | (+) | (+++) | (+) |
| **8e** | (+) | (+) | (+) | (+) |
| **8f** | (+) | (+) | (+) | (+) |
| **8g** | (+) | (+) | (+) | (+) |
| **8h** | (+) | (+++) | (+) | (+) |
| **8i** | (+) | (+) | (+) | (+) |
| **8j** | (+) | (+) | (+) | (+) |
| Meglumine antimoniate | (++) | (+) | (+) | (+) |
| $H_2O_2$ | (+++) | (+++) | (+++) | (+) |

The scale of toxicity risk ranges from low (+) to medium (++) and high (+++), calculated using Osiris software.[25]

may increase the risk of these molecules to cause a tumorigenic effect. The compound **4b** showed a high risk of mutagenic effect. Compounds **5j** and **7j** showed high risk of causing damage to the reproductive system. Other derivatives showed low risk of causing all toxic effects studied. This information is useful since the active compounds **6l**, **7e**, **8f**, and **8i** are able to be submitted to drug design development. All compounds showed lower risk of toxic effects when evaluated in comparison with the antileishmanial drug meglumine antimoniate, which showed a high risk of tumorigenic and mutagenic effects and effects on the reproductive system.

## Conclusions

Our results suggest that our synthetic methodology to synthesize fused-ring pyrazolopyridazinone has a wide application, and those rings have potential antileishmanial activity, depending on the substituents that is present in 1, 4 and 6-positions, where the phenyl substituent led to higher-activity compounds in comparison to *tert*-butyl and 4-chlorophenyl substituents. All compounds synthesized showed low cytotoxicity with good selectivity index for promastigote and amastigote forms of *L. amazonensis*.

## Experimental

### General information

Reagents were used as obtained from commercial suppliers without further purification. Solvents were dried and purified according to procedure.[26] $^1$H NMR, $^{13}$C NMR, HSQC and HMBC experiments were run on VARIAN Mercury Plus apparatus operating at $^1$H 300.06 MHz and $^{13}$C 75.46 MHz, and Bruker Avance III HD apparatus operating at $^1$H 500.13 MHz and $^{13}$C 125.77 MHz. Chemical shifts are reported in ppm using tetramethylsilane (TMS) as the internal standard for $CDCl_3$ and dimethyl sulfoxide (DMSO-$d_6$). Melting points were determined on Micro-Química apparatus model MQAPF-301. The column chromatography used was silica gel 60, with 230-400 mesh (Merck). Electrospray ionization (ESI)(+)-MS and tandem ESI(+)-MS/MS were acquired using a hybrid high-resolution and high accuracy microTof (Q-TOF, quadrupole time-of-flight) mass spectrometer (Bruker®). For ESI(+)-MS, the energy for the collision induced dissociations (CDI) was optimized for each component. For data acquisition and processing, the Q-TOF-control data analysis software (Bruker Scientific) was used. The error was calculated for all compounds in ppm. Single crystal X-ray diffraction studies: X-ray intensity data measurements

of compounds **8h** (CCDC 1576799) were collected with a Bruker APEX II CCD area-detector diffractometer and graphite-monochromated MoKα radiation. The structure was solved by direct methods using SHELXS.[27] Subsequent Fourier-difference map analyses yielded the positions of the non-hydrogen atoms. Refinements were carried out by the SHELXS package.[27] All refinements were made by full matrix least squares on $F^2$ with anisotropic displacement parameters for all non-hydrogen atoms. Hydrogen atoms were included in the refinement in calculated positions, but the atoms (of hydrogens) that are performing special bond were located in the Fourier map. The ORTEP diagram were drawn with 50% probability displacement ellipsoids using ORTEP-3 for Windows.[28]

### Parasites and cell culture

Biological activity was determined in promastigotes and axenic amastigotes of *L. amazonensis* (strain WHOM/BR/75/JOSEFA). Promastigotes were cultured in Warren (brain heart infusion, hemin, and folic acid; pH 7.0) supplemented with 10% fetal calf serum (FCS) at 25 °C. Axenic amastigotes were cultured in Schneider medium (pH 4.6) supplemented with 20% fetal bovine serum (FBS) at 32 °C.

Cytotoxicity in mammalian cells was determined in a fibroblast line (L929) and macrophages (J774A1). Fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM, pH 7.2) supplemented with 10% FBS at 37 °C in a 5% $CO_2$ atmosphere. Macrophages were cultured in RPMI-1640 (pH 7.2) medium supplemented with 10% FBS at 37 °C in a 5% $CO_2$ atmosphere.

### Dilution of compounds

Stock solutions of the compounds were prepared in DMSO and then diluted in the respective medium. The groups (controls and treated) were tested with DMSO concentrations below 1%, with concentrations that do not affect viability of the protozoa, and mammalian cells.

### Anti-proliferative assay

Promastigotes ($1 \times 10^6$ cells $mL^{-1}$) were cultured in 24-well plates in the presence and absence of different concentrations of compounds diluted in Warren medium supplemented with 10% FBS and incubated for 72 h. Axenic amastigotes ($1 \times 10^6$ cells $mL^{-1}$) were cultured in a 12-well plate in the presence and absence of different concentrations of compounds diluted in Schneider medium supplemented with 20% FBS and incubated for 72 h.

After incubation, the cell count was performed in a Neubauer chamber to determine the 50% inhibitory concentration of protozoa ($IC_{50}$).

### Cytotoxicity assay in mammalian cells

A fibroblast ($2.5 \times 10^5$ cells $mL^{-1}$) suspension was prepared in DMEM medium supplemented with 10% FBS and added to 96-well plates. Then, the plates were incubated at 37 °C in a $CO_2$ atmosphere for 24 h to obtain confluent cell growth. After incubation, cells were treated or not with different concentrations of compounds diluted in DMEM for 72 h. A macrophage ($5 \times 10^5$ cells $mL^{-1}$) suspension was prepared in RPMI-1640 medium supplemented with 10% FBS and added to 96-well plates. Then, the plates were incubated at 37 °C in a $CO_2$ atmosphere for 24 h to obtain confluent cell growth. After incubation, cells were treated or not with different concentrations of compounds diluted in RPMI-1640 for 48 h.

After treatment, medium was removed and cells were incubated with MTT (2 mg $mL^{-1}$) for 4 h. Then, DMSO was added for solubilization of the formazan and analyzed with a reading microplate reader (BIO-TEK PowerWave XS spectrophotometer) at 392 nm. The percentage of viable cells was calculated in relation to the control to determine the cytotoxic concentration to 50% of the cells ($CC_{50}$).

### Molecular modeling

Molecular modeling studies were performed on the pyrazolo[3,4-*d*]pyridazinone derivatives developed using the physicochemical properties of lipophilic, electronic and structural profiles in order to support the understanding of their antileishmanial mechanism of action and the potential toxicity effects.

The geometry of compounds was fully optimized by applying DFT B3LYP/6-31G* basis in gas phase[24] and it was used to calculate the stereoelectronic properties, $E_{HOMO}$ (highest occupied molecular orbital energy), $E_{LUMO}$ (lowest unoccupied molecular orbital energy), gap energy ($E_{LUMO} - E_{HOMO}$), and surface of electrostatic potential density (MEPs). The three-dimensional isosurfaces of the molecular MEPs at the van der Waals contact surface represented electrostatic potentials superimposed onto a surface of constant electron density (0.002 e $au^{-3}$). The color-coded isosurface values provide an indication of the overall molecular size and location of negative (red) or positive (blue) electrostatic potentials.

The compounds were also studied by applying the Lipinski's rule of five,[22] through the use of online free

web cheminformatics software Molinspiration,[23] where the following values were obtained: polar surface area (PSA), molecular weight, and number of acceptor groups and hydrogen bond donors (nON and nOHNH, respectively).

For the theoretical study of toxicity (through mutagenic effects, tumorigenic, irritant and impacts on their productive system) and the drug score and druglikeness profiles of pyrazolo[3,4-*d*]pyridazinone derivatives, the online web free Osiris Property Explorer (Actelion Pharmaceuticals Ltd, Allschwil, Switzerland, CH) computer program was used.

## General synthetic procedure and spectra data

### Diethyl 3-((dimethylamine)methylene)-2,4-dioxo-pentanedioate (**1**)

The β-enamino diketone substrate **1** was prepared according to the literature.[19]

### 5-(Ethoxycarbonyl)-4-[ethoxy(oxo)acetyl]-1-phenyl-1*H*-pyrazole (**2a**)

Pyrazole **2a** was prepared according to the literature.[19]

### 1-(*tert*-Butyl)-5-(ethoxycarbonyl)-4-[ethoxy(oxo)acetyl]-1*H*-pyrazole (**2b**)

Pyrazole **2b** was prepared according to the methodology described in the literature for the preparation of **2a**[19] with a little modification. A mixture of the compound **1** (0.271 g, 1 mmol) and *tert*-butylhydrazine hydrochloride (0.113 g, 0.9 mmol) in ethanol (2 mL) was stirred under reflux for 1 h. After, the solvent was evaporated under reduced pressure and the residue was washed with distilled water (25 mL), extracted with ethyl acetate (3 × 20 mL) and dried with anhydrous sodium sulfate. The solvent was evaporated once again under reduced pressure and the obtained residue was purified on a silica gel chromatography column, utilizing a 95:5 mixture of hexane:ethyl acetate as the eluent, affording the product as a viscous light yellow oil: 79% yield (0.211 g); $^1$H NMR (300.06 MHz, $CDCl_3$) $\delta$ 1.38 (t, 3H, *J* 7.2 Hz, O–$CH_2$–$CH_3$), 1.39 (t, 3H, *J* 7.2 Hz, O–$CH_2$–$CH_3$), 1.63 (s, 9H, *t*-Bu) 4.37 (q, 2H, *J* 7.2 Hz, O–$CH_2$–$CH_3$), 4.44 (q, 2H, *J* 7.2 Hz, O–$CH_2$–$CH_3$), 8.18 (s, 1H, H3); $^{13}$C NMR (75.46 MHz, $CDCl_3$) $\delta$ 13.8, 14.1, 29.7, 62.7, 63.3, 63.5, 119.1, 138.3, 140.5, 161.4, 162.9, 177.0; HRMS (ESI(+)): calcd. for $C_{14}H_{21}N_2O_5^+$, $[M + H]^+$: 297.1445, found 297.1469.

### 1,6-Disubstituted 4-(ethoxycarbonyl)-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**3a**,**b**,**d**)

The pyrazolopyridazinone derivatives **3a**,**b**,**d** were prepared according to the literature.[19] A mixture of compound **2** (**2a**: 0.158 g; **2b**: 0.148 g, 0.50 mmol), hydrazine (hydrazine hydrate, 80% in water: 0.022 g; 4-chlorophenylhydrazine: 0.078 g, 0.55 mmol) and acetic acid (2 mmol, 0.11 mL), was stirred under reflux in ethanol (5 mL) for 12 h. Then, the mixture was cooled to 0 °C and the solid was filtered, washed with cold ethanol (10 mL) and dried under vacuum.

### 1-(*tert*-Butyl)-4-(ethoxycarbonyl)-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**3b**)

White solid; 87% yield (0.115 g); mp 177.3-178.9 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.36 (t, 3H, *J* 7.1 Hz, O–$CH_2$–$CH_3$), 1.76 (s, 9H, *t*-Bu), 4.39 (q, 2H, *J* 7.1 Hz, O–$CH_2$–$CH_3$), 8.28 (s, 1H, H3), 13.27 (bs, 1H, NH); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 14.0 (O–$CH_2$–$\underline{C}H_3$), 29.4 ($C(\underline{C}H_3)_3$), 61.5 (O–$\underline{C}H_2$–$CH_3$), 62.4 ($\underline{C}(CH_3)_3$), 120.7 (C3a), 131.3 (C7a), 132.3 (C3), 132.4 (C4), 153.4 (C7), 162.2 (COOEt); HRMS (ESI(+)): calcd. for $C_{12}H_{17}N_4O_3^+$, $[M + H]^+$: 265.1295, found 265.1316.

### 4-(Ethoxycarbonyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**3c**)

The pyrazolopyridazinone derivative **3c** was prepared according to the literature.[21]

### 1,6-Disubstituted 4-(hydrazinecarbonyl)-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**4a-d**)

The hydrazides derivatives **4a**-**c** were prepared according to the literature.[22] A mixture of **3** (**3a**: 0.284 g; **3b**: 0.264 g; **3c**: 0.360 g; **3d**: 0.394 g, 1.0 mmol) and hydrazine monohydrate (0.84 mL, 20 mmol) was stirred under reflux in ethanol:acetonitrile (1:1, 10 mL) for 24 h. Then, the mixture was cooled to room temperature and the solvent evaporated under vacuum.

### 1-(*tert*-Butyl)-4-hydrazinecarbonyl-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**4b**)

White solid; 95% yield (0.238 g); mp 291.4-292.7 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.75 (s, 9H, *t*-Bu), 4.58 (bs, 2H, CONHN$\underline{H}_2$), 8.30 (s, 1H, H3), 9.74 (bs, 1H, CON$\underline{H}$N$H_2$), 13.03 (bs, 1H, NH); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 ($C(\underline{C}H_3)_3$), 62.2 ($\underline{C}(CH_3)_3$), 120.4 (C3a), 131.6 (C7a), 132.5 (C3), 135.4 (C4), 153.5 (C7), 161.4 ($CONHNH_2$); HRMS (ESI(+)): calcd. for $C_{10}H_{15}N_6O_2^+$, $[M + H]^+$: 251.1251, found 251.1274.

### 1,6-Disubstituted 4-[(2*E*)-*N*'-(substituted)hydrazine-carbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5**, **6**, **7**, **8e-j**, **6l**,**k**)

The *N*-acylhydrazones derivatives **5e**-**j**, **6e**-**l**, **7** and **8e**-**j** were prepared according to the literature.[21,22] A mixture

of compound **4** (**4a**: 0.081 g; **4b**: 0.075 g; **4c**: 0.104 g; **4d**: 0.114 g, 0.3 mmol), aldehyde (benzaldehyde: 0.032 g; 4-hydroxybenzaldehyde: 0.037 g; *p*-anisaldehyde: 0.041 g; 4-(dimethylamino)benzaldehyde: 0.045 g; 4-fluorobenzaldehyde: 0.037 g; 4-nitrobenzaldehyde: 0.045 g; 2-thiophenecarboxaldehyde: 0.034 g; 2,2'-bithiophene-5-carboxaldehyde: 0.058 g, 0.3 mmol) and HCl 37% (two drops) in DMSO (3 mL) was stirred at room temperature for 1 h. Then, cold distilled water (20 mL) was added to the mixture and the product was obtained as a white solid. The product was washed with cold water and dried under vacuum.

4-[(2*E*)-*N'*-(Benzylidene)hydrazinecarbonyl]-6,7-dihydro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5e**)

White solid; 95% yield (0.102 g); mp > 350 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.45-7.60 (m, 6H, PhA and PhB), 7.72-7.76 (m, 4H, PhA and PhB), 8.62 (bs, 1H, H8'), 8.66 (s, 1H, H3), 12.06 (bs, 1H, H6'), 13.33 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 119.9 (C3a), 125.5, 127.2, 128.6, 128.6, 128.9, 130.3 (PhA and PhB), 131.9 (C7a), 134.3 (PhB), 134.9 (C4), 136.5 (C3), 138.4 (PhA), 149.2 (C8'), 153.3 (C7), 158.7 (C5'); HRMS (ESI(+)): calcd. for $C_{19}H_{15}N_6O_2^+$, $[M + H]^+$: 359.1251, found 359.1270.

6,7-Dihydro-4-[(2*E*)-*N'*-(4-hydroxybenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5f**)

White solid; 94% yield (0.105 g); mp 328.4-330.6 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 6.86 (d, 2H, *J* 8.6 Hz, 4-OHC$_6$H$_4$), 7.48-7.60 (m, 5H, 4-OHC$_6$H$_4$ and Ph), 7.72-7.75 (m, 2H, Ph), 8.49 (bs, 1H, H8'), 8.65 (bs, 1H, H3), 9.98 (bs, 1H, 4-OHC$_6$H$_4$), 11.84 (bs, 1H, H6'), 13.29 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 115.8 (4-OHC$_6$H$_4$), 120.0 (C3a), 125.2, 125.5, 128.5, 128.5, 129.0 (Ph and 4-OHC$_6$H$_4$), 131.9 (C7a), 135.1 (C4), 136.6 (C3), 138.5 (Ph), 149.5 (C8'), 153.3 (C7), 158.4 (C5'), 159.6 (4-OHC$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{19}H_{15}N_6O_3^+$, $[M + H]^+$: 375.1200, found 375.1220.

6,7-Dihydro-4-[(2*E*)-*N'*-(4-methoxybenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5g**)

White solid; 95% yield (0.111 g); mp > 350 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 3.82 (s, 3H, 4-OCH$_3$C$_6$H$_4$), 7.04 (d, 2H, *J* 8.8 Hz, 4-OCH$_3$C$_6$H$_4$), 7.48-7.60 (m, 3H, Ph), 7.68 (d, 2H, *J* 8.8 Hz, 4-OCH$_3$C$_6$H$_4$), 7.71-7.76 (m, 2H, Ph), 8.54 (bs, 1H, H8'), 8.65 (s, 1H, H3), 11.92 (bs, 1H, H6'), 13.30 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 55.3 (4-OCH$_3$C$_6$H$_4$), 114.4 (4-OCH$_3$C$_6$H$_4$), 119.9 (C3a), 125.5 (Ph), 126.8 (4-OCH$_3$C$_6$H$_4$), 128.5, 128.5 (Ph), 128.8 (4-OCH$_3$C$_6$H$_4$), 131.9 (C7a), 135.1 (C4), 136.5 (C3), 138.4 (Ph), 149.0 (C8'), 153.3 (C7), 158.5 (C5'), 161.0 (4-OCH$_3$C$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{20}H_{17}N_6O_3^+$, $[M + H]^+$: 389.1357, found 389.1374.

6,7-Dihydro-4-[(2*E*)-*N'*-(4-(dimethylamino)benzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5h**)

Light yellow solid; 95% yield (0.114 g); mp 329.0-330.6 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 2.99 (s, 6H, 4-N(CH$_3$)$_2$C$_6$H$_4$), 6.77 (d, 2H, *J* 9.1 Hz, 4-N(CH$_3$)$_2$C$_6$H$_4$), 7.48-7.60 (m, 5H, Ph and 4-N(CH$_3$)$_2$C$_6$H$_4$), 7.71-7.75 (m, 2H, Ph), 8.44 (bs, 1H, H8'), 8.65 (s, 1H, H3), 11.74 (bs, 1H, H6'), 13.27 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 39.8 (4-N(CH$_3$)$_2$C$_6$H$_4$), 111.8 (4-N(CH$_3$)$_2$C$_6$H$_4$), 120.0 (C3a), 121.5 (4-N(CH$_3$)$_2$C$_6$H$_4$), 125.5, 128.5, 128.5 (Ph), 128.5 (4-N(CH$_3$)$_2$C$_6$H$_4$), 131.9 (C7a), 135.3 (C4), 136.6 (C3), 138.5 (Ph), 149.9 (C8'), 151.6 (4-N(CH$_3$)$_2$C$_6$H$_4$), 153.3 (C7), 158.2 (C5'); HRMS (ESI(+)): calcd. for $C_{21}H_{20}N_7O_2^+$, $[M + H]^+$: 402.1673, found 402.1686.

6,7-Dihydro-4-[(2*E*)-*N'*-(4-fluorobenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5i**)

White solid; 94% yield (0.106 g); mp > 350 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.32 (t, 2H, $^3J_{H-F}$ 8.9 Hz, *J* 8.9 Hz, 4-FC$_6$H$_4$), 7.48-7.60 (m, 3H, Ph), 7.72-7.76 (m, 2H, Ph), 7.80 (dd, 2H, $^4J_{H-F}$ 5.6 Hz, *J* 8.8 Hz, 4-FC$_6$H$_4$), 8.61 (bs, 1H, H8'), 8.66 (s, 1H, H3), 12.07 (bs, 1H, H6'), 13.33 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 116.0 (d, $^2J_{C-F}$ 22.0 Hz, 4-FC$_6$H$_4$), 119.9 (C3a), 125.5, 128.6, 128.6 (Ph), 129.4 (d, $^3J_{C-F}$ 8.7 Hz, 4-FC$_6$H$_4$), 130.9 (d, $^4J_{C-F}$ 2.8 Hz, 4-FC$_6$H$_4$), 131.9 (C7a), 134.9 (C4), 136.5 (C3), 138.4 (Ph), 148.0 (C8'), 153.3 (C7), 158.7 (C5'), 163.2 (d, $^1J_{C-F}$ 248.0 Hz, 4-FC$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{19}H_{14}FN_6O_2^+$, $[M + H]^+$: 377.1157, found 377.1166.

6,7-Dihydro-4-[(2*E*)-*N'*-(4-nitrobenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**5j**)

Light yellow solid; 94% yield (0.114 g); mp > 350 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.48-7.60 (m, 3H, Ph), 7.71-7.74 (m, 2H, Ph), 7.99 (d, 2H, *J* 9.0 Hz, 4-NO$_2$C$_6$H$_4$), 8.32 (d, 2H, *J* 8.9 Hz, 4-NO$_2$C$_6$H$_4$), 8.66 (s, 1H, H3), 8.71 (bs, 1H, H8'), 12.38 (bs, 1H, H6'), 13.33 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 119.9 (C3a), 124.2 (4-NO$_2$C$_6$H$_4$), 125.6 (Ph), 128.2 (4-NO$_2$C$_6$H$_4$), 128.7, 128.7 (Ph), 131.9 (C7a), 134.7 (C4), 136.5 (C3), 138.5 (Ph), 140.6 (4-NO$_2$C$_6$H$_4$), 146.8 (C8'), 148.0 (4-NO$_2$C$_6$H$_4$), 153.4 (C7), 159.1 (C5'); HRMS (ESI(+)): calcd. for $C_{19}H_{14}N_7O_4^+$, $[M + H]^+$: 404.1102, found 404.1093.

4-[(2*E*)-*N*'-(Benzylidene)hydrazinecarbonyl]-1-(*tert*-butyl)-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6e**)

White solid; 95% yield (0.096 g); mp 304.8-306.9 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.79 (s, 9H, *t*-Bu), 7.43-7.52 (m, 3H, Ph), 7.71-7.75 (m, 2H, Ph), 8.39 (s, 1H, H3), 8.59 (bs, 1H, H8'), 12.00 (bs, 1H, H6'), 13.24 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 62.4 ($\underline{C}(CH_3)_3$), 120.4 (C3a), 127.1, 128.9, 130.2 (Ph), 131.7 (C7a), 132.6 (C3), 134.3 (Ph), 135.1 (C4), 149.0 (C8'), 153.7 (C7), 158.8 (C5'); HRMS (ESI(+)): calcd. for $C_{17}H_{19}N_6O_2^+$, $[M + H]^+$: 339.1564, found 339.1568.

1-(*tert*-Butyl)-6,7-dihydro-4-[(2*E*)-*N*'-(4-hydroxybenzylidene) hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6f**)

Light yellow solid; 95% yield (0.101 g); mp 310.4-312.8 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.78 (s, 9H, *t*-Bu), 6.85 (d, 2H, *J* 8.7 Hz, 4-OHC$_6\underline{H}_4$), 7.56 (d, 2H, *J* 8.7 Hz, 4-OHC$_6\underline{H}_4$), 8.37 (s, 1H, H3), 8.46 (bs, 1H, H8'), 9.96 (bs, 1H, 4-O$\underline{H}$C$_6$H$_4$), 11.78 (bs, 1H, H6'), 13.19 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 62.3 ($\underline{C}(CH_3)_3$), 115.8 (4-OHC$_6$H$_4$), 120.5 (C3a), 125.2, 128.9 (4-OHC$_6$H$_4$), 131.7 (C7a), 132.6 (C3), 135.3 (C4), 149.3 (C8'), 153.6 (C7), 158.5 (C5'), 159.6 (4-OHC$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{17}H_{19}N_6O_3^+$, $[M + H]^+$: 355.1513, found 355.1515.

1-(*tert*-Butyl)-6,7-dihydro-4-[(2*E*)-*N*'-(4-methoxybenzylidene)hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6g**)

White solid; 95% yield (0.105 g); mp 271.5-272.0 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.79 (s, 9H, *t*-Bu), 3.82 (s, 3H, 4-OC$\underline{H}_3$C$_6$H$_4$), 7.04 (d, 2H, *J* 8.8 Hz, 4-OCH$_3$C$_6\underline{H}_4$), 7.67 (d, 2H, *J* 8.9 Hz, 4-OCH$_3$C$_6\underline{H}_4$), 8.38 (s, 1H, H3), 8.52 (bs, 1H, H8'), 11.86 (bs, 1H, H6'), 13.21 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 55.3 (4-O$\underline{C}$H$_3$C$_6$H$_4$), 62.3 ($\underline{C}(CH_3)_3$), 114.4 (4-OCH$_3\underline{C}_6$H$_4$), 120.5 (C3a), 126.8, 128.8 (4-OCH$_3\underline{C}_6$H$_4$), 131.7 (C7a), 132.6 (C3), 135.2 (C4), 148.9 (C8'), 153.7 (C7), 158.6 (C5'), 161.0 (4-OCH$_3\underline{C}_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{18}H_{21}N_6O_3^+$, $[M + H]^+$: 369.1670, found 369.1674.

1-(*tert*-Butyl)-6,7-dihydro-4-[(2*E*)-*N*'-(4-(dimethylamino) benzylidene)hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6h**)

Yellow solid; 96% yield (0.110 g); mp 270.4-272.4 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.78 (s, 9H, *t*-Bu), 2.99 (s, 6H, 4-N(C$\underline{H}_3)_2$C$_6$H$_4$), 6.77 (d, 2H, *J* 8.9 Hz, 4-N(CH$_3)_2$C$_6\underline{H}_4$), 7.54 (d, 2H, *J* 8.9 Hz, 4-N(CH$_3)_2$C$_6\underline{H}_4$), 8.37 (s, 1H, H3), 8.41 (bs, 1H, H8'), 11.67 (bs, 1H, H6'), 13.18 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 39.8 (4-N($\underline{C}H_3)_2$C$_6$H$_4$), 62.3 ($\underline{C}(CH_3)_3$), 111.8 (4-N(CH$_3)_2\underline{C}_6$H$_4$), 120.5 (C3a), 121.5, 128.5 (4-N(CH$_3)_2\underline{C}_6$H$_4$), 131.7 (C7a), 132.6 (C3), 135.4 (C4), 149.8 (C8'), 151.6 (4-N(CH$_3)_2\underline{C}_6$H$_4$), 153.6 (C7), 158.3 (C5'); HRMS (ESI(+)): calcd. for $C_{19}H_{24}N_7O_2^+$, $[M + H]^+$: 382.1986, found 382.1998.

1-(*tert*-Butyl)-6,7-dihydro-4-[(2*E*)-*N*'-(4-fluorobenzylidene) hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6i**)

White solid; 94% yield (0.100 g); mp 314.4-315.4 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.79 (s, 9H, *t*-Bu), 7.32 (t, 2H, $^3J_{H\text{-}F}$ 8.9 Hz, *J* 8.9 Hz, 4-FC$_6\underline{H}_4$), 7.79 (dd, 2H, $^4J_{H\text{-}F}$ 5.7 Hz, *J* 8.7 Hz, 4-FC$_6\underline{H}_4$), 8.39 (s, 1H, H3), 8.59 (bs, 1H, H8'), 12.01 (bs, 1H, H6'), 13.24 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 62.3 ($\underline{C}(CH_3)_3$), 116.0 (d, $^2J_{C\text{-}F}$ 22.0 Hz, 4-FC$_6$H$_4$), 120.4 (C3a), 129.3 (d, $^3J_{C\text{-}F}$ 8.6 Hz, 4-FC$_6$H$_4$), 130.9 (d, $^4J_{C\text{-}F}$ 3.1 Hz, 4-FC$_6$H$_4$), 131.7 (C7a), 132.5 (C3), 135.1 (C4), 147.9 (C8'), 153.7 (C7), 158.8 (C5'), 163.2 (d, $^1J_{C\text{-}F}$ 247.8 Hz, 4-FC$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{17}H_{18}FN_6O_2^+$, $[M + H]^+$: 357.1470, found 357.1473.

1-(*tert*-Butyl)-6,7-dihydro-4-[(2*E*)-*N*'-(4-nitrobenzylidene) hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6j**)

Light yellow solid; 94% yield (0.108 g); mp 317.4-319.5 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 1.79 (s, 9H, *t*-Bu), 7.98 (d, 2H, *J* 8.9 Hz, 4-NO$_2$C$_6\underline{H}_4$), 8.31 (d, 2H, *J* 8.8 Hz, 4-NO$_2$C$_6\underline{H}_4$), 8.39 (s, 1H, H3), 8.70 (bs, 1H, H8'), 12.30 (bs, 1H, H6'), 13.28 (bs, 1H, H6); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 62.4 ($\underline{C}(CH_3)_3$), 120.4 (C3a), 124.1, 128.0 (4-NO$_2$C$_6$H$_4$), 131.7 (C7a), 132.5 (C3), 134.8 (C4), 140.6 (4-NO$_2$C$_6$H$_4$), 146.5 (C8'), 147.9 (4-NO$_2$C$_6$H$_4$), 153.7 (C7), 159.1 (C5'); HRMS (ESI(+)): calcd. for $C_{17}H_{18}N_7O_4^+$, $[M + H]^+$: 384.1415, found 384.1385.

1-(*tert*-Butyl)-6,7-dihydro-4-[(2*E*)-*N*'-(2-thienylmethylene) hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6k**)

White solid; 95% yield (0.098 g); mp 319.2-322.1 °C; $^1$H NMR (500.13 MHz, DMSO-$d_6$) $\delta$ 1.77 (s, 9H, *t*-Bu), 7.15 (dd, 1H, *J* 5.1, 3.6 Hz, 2-thienyl), 7.44 (dd, 1H, *J* 3.7, 1.1 Hz, 2-thienyl), 7.69 (dd, 1H, *J* 5.0 Hz, 2-thienyl), 8.36 (s, 1H, H3), 8.76 (bs, 1H, H8'), 11.99 (bs, 1H, H6'), 13.21 (bs, 1H, H6); $^{13}$C NMR (125.77 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 62.4 ($\underline{C}(CH_3)_3$), 120.4 (C3a), 127.9, 129.2, 131.1 (2-thienyl), 131.7 (C7a), 132.6 (C3), 135.1 (C4), 139.1 (2-thienyl), 144.0 (C8'), 153.7 (C7), 158.7 (C5'); HRMS (ESI(+)): calcd. for $C_{15}H_{17}N_6O_2S^+$, $[M + H]^+$: 345.1128, found 345.1130.

4-[(2*E*)-*N*'-(2,2'-Bithienyl-5-methylene)hydrazinecarbonyl]-1-(*tert*-butyl)-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**6l**)

Yellow solid; 96% yield (0.123 g); mp 223.2-225.3 °C; $^1$H NMR (500.13 MHz, DMSO-$d_6$) $\delta$ 1.77 (s, 9H, *t*-Bu), 7.13 (dd, 1H, *J* 5.1, 3.6 Hz, 2,2'-bithienyl), 7.32 (d, 1H, *J* 3.8 Hz, 2,2'-bithienyl), 7.40 (d, 1H, *J* 3.9 Hz, 2,2'-bithienyl), 7.44 (dd, 1H, *J* 3.7, 1.2 Hz, 2,2'-bithienyl), 7.59 (dd, 1H, *J* 5.1, 1.2 Hz, 2,2'-bithienyl), 8.37 (s, 1H, H3), 8.71 (bs, 1H, H8'), 12.05 (bs, 1H, H6'), 13.21 (bs, 1H, H6); $^{13}$C NMR (125.77 MHz, DMSO-$d_6$) $\delta$ 29.5 (C($\underline{C}H_3)_3$), 62.4 ($\underline{C}(CH_3)_3$), 120.4 (C3a), 124.3, 125.2, 126.6, 128.6 (2,2'-bithienyl), 131.7 (C7a), 132.2 (2,2'-bithienyl), 132.6 (C3), 135.1 (C4), 136.1, 137.8, 138.9 (2,2'-bithienyl), 143.6 (C8'), 153.7 (C7), 158.6 (C5'); HRMS (ESI(+)): calcd. for $C_{19}H_{19}N_6O_2S_2^+$, [M + H]$^+$: 427.1005, found 427.1008.

4-[(2*E*)-*N*'-(Benzylidene)hydrazinecarbonyl]-1,6-diphenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**7e**)

Light yellow solid; 94% yield (0.122 g); mp 259.3-260.6 °C; $^1$H NMR (300.06 MHz, $CDCl_3$) $\delta$ 7.36-7.58 (m, 1H, PhA, B and C), 7.66-7.71 (m, 2H, PhA), 7.75-7.79 (m, 2H, PhC), 8.23 (bs, 1H, H8'), 8.87 (s, 1H, H3), 10.18 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, $CDCl_3$) $\delta$ 120.0 (C3a), 125.7, 126.5, 127.9, 128.7, 128.9, 128.9, 129.1, 129.1, 130.9, 133.4 (PhA, B and C), 132.1 (C7a), 134.4 (C4), 137.5 (C3), 138.5, 140.8 (PhA and B), 149.4 (C8'), 152.6 (C7), 157.9 (C5'); HRMS (ESI(+)): calcd. for $C_{25}H_{19}N_6O_2^+$, [M + H]$^+$: 435.1564, found 435.1576.

1,6-Diphenyl-4-[(2*E*)-*N*'-(4-hydroxybenzylidene)hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**7f**)

Light yellow solid; 95% yield (0.128 g); mp > 350 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 6.85 (d, 2H, *J* 8.6 Hz, 4-OHC$_6\underline{H}_4$), 7.43-7.58 (m, 8H, 4-OHC$_6\underline{H}_4$, PhA and B), 7.68-7.75 (m, 4H, PhA and B), 8.47 (bs, 1H, H8'), 8.69 (s, 1H, H3), 9.98 (bs, 1H, 4-O$\underline{H}$C$_6$H$_4$), 11.72 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 115.8 (4-OHC$_6$H$_4$), 119.6 (C3a), 125.1 (4-OHC$_6$H$_4$), 125.7, 126.8, 128.2, 128.5, 128.6, 128.7 (PhA and B), 129.0 (4-OHC$_6$H$_4$), 132.3 (C7a), 134.6 (C4), 136.5 (C3), 138.4, 140.9 (PhA and B), 149.9 (C8'), 152.0 (C7), 158.1 (C5'), 159.7 (4-OHC$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{25}H_{19}N_6O_3^+$, [M + H]$^+$: 451.1513, found 451.1514.

1,6-Diphenyl-4-[(2*E*)-*N*'-(4-methoxybenzylidene)hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**7g**)

White solid; 96% yield (0.134 g); mp 247.3-248.6 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3H, 4-OC$\underline{H}_3$C$_6$H$_4$), 7.04 (d, 2H, *J* 8.8 Hz, 4-OCH$_3$C$_6\underline{H}_4$), 7.44-7.58 (m, 6H, PhA and B), 7.65-7.76 (m, 6H, 4-OCH$_3$C$_6\underline{H}_4$, PhA and B), 8.52 (bs, 1H, H8'), 8.69 (s, 1H, H3), 11.79 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 55.3 (4-O$\underline{C}$H$_3$C$_6$H$_4$), 114.4 (4-OCH$_3\underline{C}_6$H$_4$), 119.6 (C3a), 125.7, 126.8, 128.2, 128.5, 128.6, 128.7 (PhA and B), 126.7, 128.9 (4-OCH$_3\underline{C}_6$H$_4$), 132.3 (C7a), 134.5 (C4), 136.5 (C3), 138.4, 140.9 (PhA and B), 149.5 (C8'), 152.0 (C7), 158.2 (C5'), 161.0 (4-OCH$_3\underline{C}_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{26}H_{21}N_6O_3^+$, [M + H]$^+$: 465.1670, found 465.1678.

4-[(2*E*)-*N*'-(4-(Dimethylamino)benzylidene)hydrazinecarbonyl]-1,6-diphenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**7h**)

Yellow solid; 96% yield (0.137 g); mp 267.5-268.9 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 2.98 (s, 6H, 4-N($C\underline{H}_3$)$_2$C$_6$H$_4$), 6.76 (d, 2H, *J* 9.0 Hz, 4-N(CH$_3$)$_2$C$_6\underline{H}_4$), 7.43-7.58 (m, 8H, 4-N(CH$_3$)$_2$C$_6\underline{H}_4$, PhA and B), 7.68-7.75 (m, 4H, PhA and B), 8.41 (bs, 1H, H8'), 8.68 (s, 1H, H3), 11.62 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 39.5 (4-N($\underline{C}$H$_3$)$_2$C$_6$H$_4$), 111.8 (4-N(CH$_3$)$_2\underline{C}_6$H$_4$), 119.7 (C3a), 121.3 (4-N(CH$_3$)$_2\underline{C}_6$H$_4$), 125.7, 126.8, 128.2, 128.5, 128.5, 128.6 (PhA and B), 128.6 (4-N(CH$_3$)$_2\underline{C}_6$H$_4$), 132.2 (C7a), 134.8 (C4), 136.5 (C3), 138.4, 140.9 (PhA and B), 150.4 (C8'), 151.7 (4-N(CH$_3$)$_2\underline{C}_6$H$_4$), 152.0 (C7), 157.9 (C5'); HRMS (ESI(+)): calcd. for $C_{27}H_{24}N_7O_2^+$, [M + H]$^+$: 478.1986, found 478.1985.

1,6-Diphenyl-4-[(2*E*)-*N*'-(4-fluorobenzylidene)hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**7i**)

White solid; 95% yield (0.129 g); mp 251.2-252.2 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.31 (t, 2H, $^3J_{H\text{-}F}$ 8.9 Hz, *J* 8.9 Hz, 4-FC$_6$H$_4$), 7.43-7.58 (m, 6H, PhA and B), 7.68-7.75 (m, 4H, PhA and B), 7.79 (dd, 2H, $^4J_{H\text{-}F}$ 5.6 Hz, *J* 8.9 Hz, 4-FC$_6$H$_4$), 8.58 (bs, 1H, H8'), 8.69 (s, 1H, H3), 11.93 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 116.0 (d, $^2J_{C\text{-}F}$ 22.0 Hz, 4-FC$_6$H$_4$), 119.6 (C3a), 125.7, 126.8, 128.3, 128.5, 128.6, 128.7 (PhA and B), 129.4 (d, $^3J_{C\text{-}F}$ 8.7 Hz, 4-FC$_6$H$_4$), 130.8 (d, $^4J_{C\text{-}F}$ 2.9 Hz, 4-FC$_6$H$_4$), 132.2 (C7a), 134.3 (C4), 136.5 (C3), 138.4, 140.9 (PhA and B), 148.5 (C8'), 152.0 (C7), 158.4 (C5'), 163.3 (d, $^1J_{C\text{-}F}$ 248.2 Hz, 4-FC$_6$H$_4$); HRMS (ESI(+)): calcd. for $C_{25}H_{18}FN_6O_2^+$, [M + H]$^+$: 453.147, found 453.1484.

1,6-Diphenyl-4-[(2*E*)-*N*'-(4-nitrobenzylidene)hydrazinecarbonyl]-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**7j**)

White solid; 94% yield (0.135 g); mp 277.6-278.2 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.45-7.58 (m, 6H, PhA and B), 7.68-7.76 (m, 4H, PhA and B), 7.98 (d, 2H, *J* 8.9 Hz, 4-NO$_2$C$_6$H$_4$), 8.32 (d, 2H, *J* 8.9 Hz, 4-NO$_2$C$_6$H$_4$), 8.70 (bs, 1H, H8'), 8.71 (s, 1H, H3), 12.22 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 119.6 (C3a), 124.1 (4-NO$_2$C$_6$H$_4$), 125.7, 126.9, 128.3, 128.5, 128.6, 128.7 (PhA and B), 128.1 (4-NO$_2$C$_6$H$_4$), 132.3 (C7a), 134.1 (C4), 136.4 (C3), 138.4,

140.9 (PhA and B), 140.4 (4-$NO_2C_6H_4$), 147.1 (C8'), 148.0 (4-$NO_2C_6H_4$), 152.0 (C7), 158.8 (C5'); HRMS (ESI(+)): calcd. for $C_{25}H_{18}N_7O_4^+$, $[M + H]^+$: 480.1415, found 480.1422.

4-[(2*E*)-*N*'-(Benzylidene)hydrazinecarbonyl]-6-(4-chlorophenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**8e**)

White solid; 96% yield (0.135 g); mp 255.7-257.5 °C; $^1$H NMR (500.13 MHz, $CDCl_3$) $\delta$ 7.37-7.42 (m, 3H, PhB), 7.44-7.51 (m, 5H, PhA and 4-$ClC_6H_4$), 7.54 (d, 2H, *J* 8.8 Hz, 4-$ClC_6H_4$), 7.66-7.68 (m, 2H, PhA), 7.75-7.76 (m, 2H, PhB), 8.26 (bs, 1H, H8'), 8.85 (s, 1H, H3), 10.17 (bs, 1H, H6'); $^{13}$C NMR (125.77 MHz, $CDCl_3$) $\delta$ 120.0 (C3a), 125.7, 127.8 (PhA and 4-$ClC_6H_4$), 127.9 (PhB), 128.8, 128.9, 129.2 (PhA and 4-$ClC_6H_4$), 129.2, 131.0 (PhB), 132.0 (C7a), 133.4 (PhB), 134.7 (4-$ClC_6H_4$), 134.8 (C4), 137.6 (C3), 138.5, 139.3 (PhA and 4-$ClC_6H_4$), 149.6 (C8'), 152.5 (C7), 157.8 (C5'); HRMS (ESI(+)): calcd. for $C_{25}H_{18}ClN_6O_2^+$, $[M + H]^+$: 469.1174, found 469.1169.

6-(4-Chlorophenyl)-4-[(2*E*)-*N*'-(4-hydroxybenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**8f**)

Yellow solid; 94% yield (0.137 g); mp > 350 °C; $^1$H NMR (500.13 MHz, DMSO-$d_6$) $\delta$ 6.85 (d, 2H, *J* 8.6 Hz, 4-$OHC_6\underline{H}_4$), 7.49-7.58 (m, 5H, 4-$OHC_6\underline{H}_4$ and Ph), 7.61 (d, 2H, *J* 8.7 Hz, 4-$ClC_6H_4$), 7.73-7.74 (m, 2H, Ph), 7.77 (d, 2H, *J* 8.7 Hz, 4-$ClC_6H_4$), 8.46 (bs, 1H, H8'), 8.69 (s, 1H, H3), 9.98 (bs, 1H, 4-$O\underline{H}C_6H_4$), 11.73 (bs, 1H, H6'); $^{13}$C NMR (125.77 MHz, DMSO-$d_6$) $\delta$ 115.8 (4-$OHC_6H_4$), 119.6 (C3a), 125.1 (4-$OHC_6H_4$), 125.6, 128.5, 128.5, 128.6, 128.7 (Ph and 4-$ClC_6H_4$), 129.0 (4-$OHC_6H_4$), 132.1 (C7a), 132.6 (4-$ClC_6H_4$), 134.8 (C4), 136.5 (C3), 138.4, 139.7 (Ph and 4-$ClC_6H_4$), 149.9 (C8'), 151.9 (C7), 158.0 (C5'), 159.7 (4-$OHC_6H_4$); HRMS (ESI(+)): calcd. for $C_{25}H_{18}ClN_6O_3^+$, $[M + H]^+$: 485.1123, found 485.1119.

6-(4-Chlorophenyl)-4-[(2*E*)-*N*'-(4-methoxybenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**8g**)

White solid; 94% yield (0.140 g); mp 154.6-155.8 °C; $^1$H NMR (300.06 MHz, $CDCl_3$) $\delta$ 3.83 (s, 3H, 4-$OC\underline{H}_3C_6H_4$), 6.91 (d, 2H, *J* 8.8 Hz, 4-$OCH_3C_6\underline{H}_4$), 7.44-7.57 (m, 7H, Ph and 4-$ClC_6H_4$), 7.65-7.73 (m, 4H, 4-$OCH_3C_6\underline{H}_4$ and Ph), 8.18 (bs, 1H, H8'), 8.86 (s, 1H, H3), 10.06 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, $CDCl_3$) $\delta$ 55.5 (4-$O\underline{C}H_3C_6H_4$), 114.4 (4-$OCH_3\underline{C}_6H_4$), 120.0 (C3a), 125.7, 127.8, 128.8, 129.1, 129.2 (Ph and 4-$ClC_6H_4$), 126.0, 129.6 (4-$OCH_3\underline{C}_6H_4$), 131.9 (C7a), 134.6 (4-$ClC_6H_4$), 134.9 (C4), 137.6 (C3), 138.5, 139.3 (Ph and 4-$ClC_6H_4$), 149.4 (C8'), 152.5 (C7), 157.6 (C5'), 162.0 (4-$OCH_3\underline{C}_6H_4$); HRMS (ESI(+)): calcd. for $C_{26}H_{20}ClN_6O_3^+$, $[M + H]^+$: 499.1280, found 499.1280.

6-(4-Chlorophenyl)-4-[(2*E*)-*N*'-(4-(dimethylamino)benzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**8h**)

Yellow solid; 96% yield (0.147 g); mp 245.2-246.7 °C; $^1$H NMR (500.13 MHz, $CDCl_3$) $\delta$ 2.99 (s, 6H, 4-$N(C\underline{H}_3)_2C_6H_4$), 6.63 (d, 2H, *J* 9.0 Hz, 4-$N(CH_3)_2C_6\underline{H}_4$), 7.43-7.50 (m, 5H, 4-$ClC_6H_4$ and Ph), 7.55 (d, 2H, *J* 8.8 Hz, 4-$ClC_6H_4$), 7.59 (d, 2H, *J* 8.8 Hz, 4-$N(CH_3)_2C_6\underline{H}_4$), 7.66-7.68 (m, 2H, Ph), 8.08 (bs, 1H, H8'), 8.85 (s, 1H, H3), 9.99 (bs, 1H, H6'); $^{13}$C NMR (125.77 MHz, $CDCl_3$) $\delta$ 40.2 (4-$N(\underline{C}H_3)_2C_6H_4$), 111.7 (4-$N(CH_3)_2\underline{C}_6H_4$), 120.1 (C3a), 120.8 (4-$N(CH_3)_2\underline{C}_6H_4$), 125.7, 127.8, 128.7, 129.0, 129.2 (Ph and 4-$ClC_6H_4$), 129.5 (4-$N(CH_3)_2\underline{C}_6H_4$), 132.0 (C7a), 134.5 (4-$ClC_6H_4$), 135.2 (C4), 137.7 (C3), 138.6, 139.4 (Ph and 4-$ClC_6H_4$), 150.3 (C8'), 152.2 (4-$N(CH_3)_2\underline{C}_6H_4$), 152.5 (C7), 157.3 (C5'); HRMS (ESI(+)): calcd. for $C_{27}H_{23}ClN_7O_2^+$, $[M + H]^+$: 512.1596, found 512.1593.

6-(4-Chlorophenyl)-4-[(2*E*)-*N*'-(4-fluorobenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**8i**)

White solid; 95% yield (0.139 g); mp 279.9-280.2 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.32 (t, 2H, $^3J_{H\text{-}F}$ 8.9 Hz, *J* 8.9 Hz, 4-$FC_6H_4$), 7.50-7.63 (m, 5H, Ph and 4-$ClC_6H_4$), 7.72-7.82 (m, 6H, Ph, 4-$ClC_6H_4$ and 4-$FC_6H_4$), 8.58 (bs, 1H, H8'), 8.69 (s, 1H, H3), 11.94 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 116.0 (d, $^2J_{C\text{-}F}$ 22.0 Hz, 4-$FC_6H_4$), 119.6 (C3a), 125.7, 128.5, 128.5, 128.6, 128.7 (Ph and 4-$ClC_6H_4$), 129.4 (d, $^3J_{C\text{-}F}$ 8.8 Hz, 4-$FC_6H_4$), 130.7 (d, $^4J_{C\text{-}F}$ 3.1 Hz, 4-$FC_6H_4$), 132.2 (C7a), 134.5 (C4), 136.5 (C3), 132.7, 138.4, 139.7 (Ph and 4-$ClC_6H_4$), 148.5 (C8'), 152.0 (C7), 158.3 (C5'), 163.3 (d, $^1J_{C\text{-}F}$ 248.1 Hz, 4-$FC_6H_4$); HRMS (ESI(+)): calcd. for $C_{25}H_{17}ClFN_6O_2^+$, $[M + H]^+$: 487.1080, found 487.1082.

6-(4-Chlorophenyl)-4-[(2*E*)-*N*'-(4-nitrobenzylidene)hydrazinecarbonyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyridazin-7-one (**8j**)

Light yellow solid; 96% yield (0.148 g); mp 303.5-304.3 °C; $^1$H NMR (300.06 MHz, DMSO-$d_6$) $\delta$ 7.48-7.58 (m, 3H, Ph), 7.62 (d, 2H, *J* 8.8 Hz, 4-$ClC_6H_4$), 7.71-7.79 (m, 4H, Ph and 4-$ClC_6H_4$), 7.98 (d, 2H, *J* 8.9 Hz, 4-$NO_2C_6H_4$), 8.31 (d, 2H, *J* 8.9 Hz, 4-$NO_2C_6H_4$), 8.69 (bs, 2H, H3 and H8'), 12.21 (bs, 1H, H6'); $^{13}$C NMR (75.46 MHz, DMSO-$d_6$) $\delta$ 119.5 (C3a), 124.2 (4-$NO_2C_6H_4$), 125.7, 128.5, 128.6, 128.7, 128.7 (Ph and 4-$ClC_6H_4$), 128.2 (4-$NO_2C_6H_4$), 132.2 (C7a), 134.3 (C4), 136.5 (C3), 132.7, 138.3, 139.7 (Ph and 4-$ClC_6H_4$), 140.4 (4-$NO_2C_6H_4$), 147.2 (C8'), 148.0 (4-$NO_2C_6H_4$), 152.0 (C7), 158.7 (C5'); HRMS (ESI(+)): calcd. for $C_{25}H_{17}ClN_7O_4^+$, $[M + H]^+$: 514.1025, found 514.1017.

## Supplementary Information

Crystallographic data for compound **8h** in this work were deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1576799. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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