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New 1*H*-Benzo[*f*]indazole-4,9-diones Conjugated with C-Protected Amino Acids and Other Derivatives: Synthesis and *in Vitro* Antiproliferative Evaluation

Aurora Molinari [1,*], Alfonso Oliva [1,*], Marlene Arismendi-Macuer [1], Leda Guzmán [1], Mauricio Fuentealba [1], Marcela Knox [1], Raúl Vinet [2,3] and Arturo San Feliciano [4]

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1 Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso 2373223, Chile; m.arismendi.m@gmail.com (M.A.-M.); leda.guzman@ucv.cl (L.G.); mauricio.fuentealba@ucv.cl (M.F.); marcela.knox@gmail.com (M.K.)
2 Facultad de Farmacia, Universidad de Valparaíso, Valparaíso 2360102, Chile; raul.vinet@uv.cl
3 Centro Regional de Estudios en Alimentos y Salud (CREAS), Valparaíso 2362696, Chile
4 Facultad de Farmacia, Departamento de Química Farmacéutica, CIETUS, IBSAL, Universidad de Salamanca, Salamanca 37007, Spain; asf@usal.es
* Correspondence: amolinar@ucv.cl (A.M.); aoliva@ucv.cl (A.O.); Tel.: +56-032-227-4938 (A.M.); Fax: +56-032-227-4910 (A.M.)

Abstract: 1*H*-Benzo[*f*]indazole-4,9-dione derivatives conjugated with C-protected amino acids (glycine, L-alanine, L-phenylalanine and L-glutamic acid) **6a–l** were prepared by chemically modifying the prenyl substituent of 3-methyl-7-(4-methylpent-3-enyl)-1*H*-benzo[*f*]indazole-4,9-dione **2** through epoxidation, degradative oxidation, oxidation and N-acyl condensation reactions. The chemical structures of the synthesized compounds were elucidated by analyzing their IR, ^{1}H-NMR and ^{13}C-NMR spectral data together with elemental analysis for carbon, hydrogen and nitrogen. The preliminary *in vitro* antiproliferative activity of the synthesized derivatives was evaluated on KATO-III and MCF-7 cell lines using a cell proliferation assay. The majority of the derivatives exhibited significant antiproliferative activity with IC_{50} values ranging from 25.5 to 432.5 μM. These results suggest that 1*H*-benzo[*f*]indazole-4,9-dione derivatives are promising molecules to be researched for developing new anticancer agents.

Keywords: 1,4-naphthoquinone; 1*H*-benzoindazole; pyrazole; amino acid

1. Introduction

A considerable number of naturally occurring and synthetic compounds that contain a 1,4-quinone moiety have been investigated for antitumor activity [1–5]. These compounds generate a quinone/hydroquinone one-electron redox process that inhibits mitochondrial electron transport and decouples oxidative phosphorylation [5]. Additionally, they act as topoisomerase inhibitors via DNA intercalation or as alkylating agents that add across both strands of the double helix, thereby leading to cancer cell death. Furthermore, it has been suggested that the quinone-induced inhibition of cancer cell growth can be attributed to the generation of reactive oxygen species (ROS) after redox cycling [1–7]. 1*H*-Indazolediones are nitrogen-containing heterocyclic 1,4-quinones that possess interesting chemical and biological properties, including antitumor activities against Ehrlich ascites carcinoma growth in male CF_1 mice, P-388 lymphocytic leukemia in male BDF_1 mice and L1210 murine leukemia cells [8–18]. In the literature, there are various synthetic methods for preparing 1*H*-indazoles [8], whereas 1*H*-indazole-4,7 and 4,9-diones are synthesized via the 1,3-dipolar cycloaddition of diazomethanes to 1,4-quinones [10,19–22]. We have recently reported the synthesis of unsubstituted

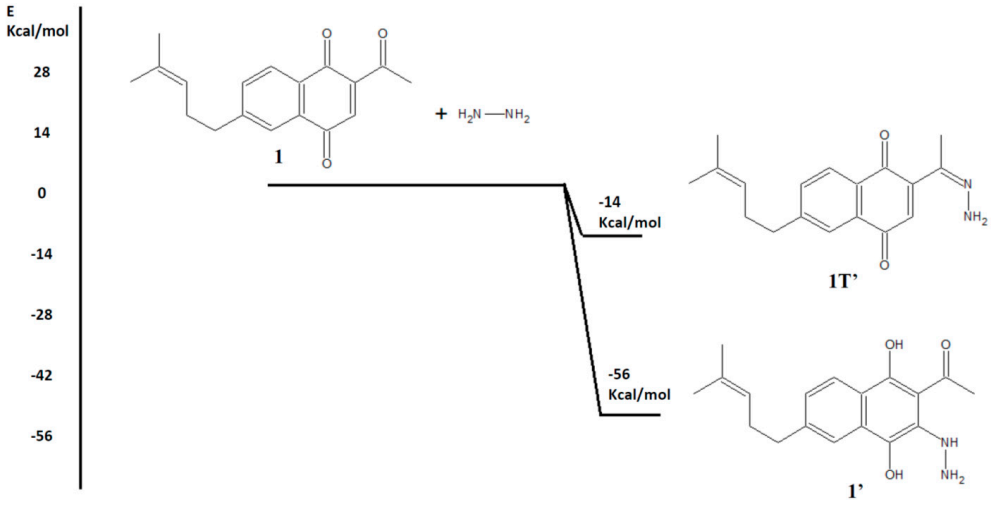
and *N*-substituted 3-methyl-7-(4-methylpent-3-enyl)-1*H*-benzo[*f*]indazole-4,9-diones **2** from the reaction of 2-acetyl-6-(4-methylpent-3-enyl)-1,4-naphthoquinone **1** with hydrazine or substituted hydrazines [23]. The objective of this work was to continue previous research on the design and synthesis of new potentially cytotoxic 1,4-naphthoquinone compounds [24,25] while taking into account the enhanced cytotoxic effect that has been observed in drugs or compounds conjugated with amino acids [26,27]. Therefore, we prepared twenty one new 1*H*-benzo[*f*]indazole-4,9-dione compounds conjugated with glycine and the L-type amino acids alanine, phenylalanine, and glutamic acid **6a**–**l**, as well as the epoxides **3a**–**c**, aldehydes **4a**–**c** and carboxylic acids **5a**–**c**, by chemically modifying the prenyl 7-(4-methylpent-3-enyl) substituent of **2**. Additionally, we evaluated the antiproliferative activity of these new compounds on KATO-III and MCF-7 cell lines of human gastric cancer and human breast cancer, respectively.

2. Results and Discussion

2.1. Chemistry

The new derivatives were prepared using 1*H*-benzo[*f*]indazole-4,9-diones **2** (R = -H; -CH_2CH_2OH; -CH_2CH_2OAc) as starting substrates, which were conveniently obtained through a direct cyclization reaction of 2-acetyl-6-(4-methylpent-3-enyl)-1,4-naphthoquinone **1** with hydrazines, triethylamine and catalytic glacial acetic acid [23]. The first step of this reaction could be (i) the conjugate addition of the hydrazine to the 1,4-naphthoquinone unit to afford the 3-substituted 1,4-hydroquinone compound **1′** or (ii) the condensation reaction between the acetyl group and hydrazine to form the corresponding hydrazone **1T′**.

To rationalize the possible products, we performed full geometry optimizations of the structures using preliminary density functional theory (DFT) calculations (see the Experimental Section). With the aim of exploring the reactants and possible products of the first step of this reaction from a thermodynamics perspective, we subtracted the total bonding energies of the products from the total bonding energies of the reactants. The comparison between these energy changes indicated that the formation of **1′** is 4-fold more favorable than the formation of **1T′** (Scheme 1). Moreover, the HOMO-LUMO gaps of compounds **1′** and **1T′** were 2.28 eV and 1.39 eV, respectively, which indicates that the most stable derivative is compound **1′** (Figure 1). These results suggest that cyclization must initially occur by the conjugate addition to afford the 3-substituted 1,4-hydroquinone compound **1′**, but a complete transition state (TS) search and intrinsic reaction coordinate (IRC) study are needed to confirm this hypothesis. For this reason, our research group is currently working to obtain a full reaction pathway for all reaction steps.



Scheme 1. Comparison between reaction energies of **1** to **1′** and **1T′**.

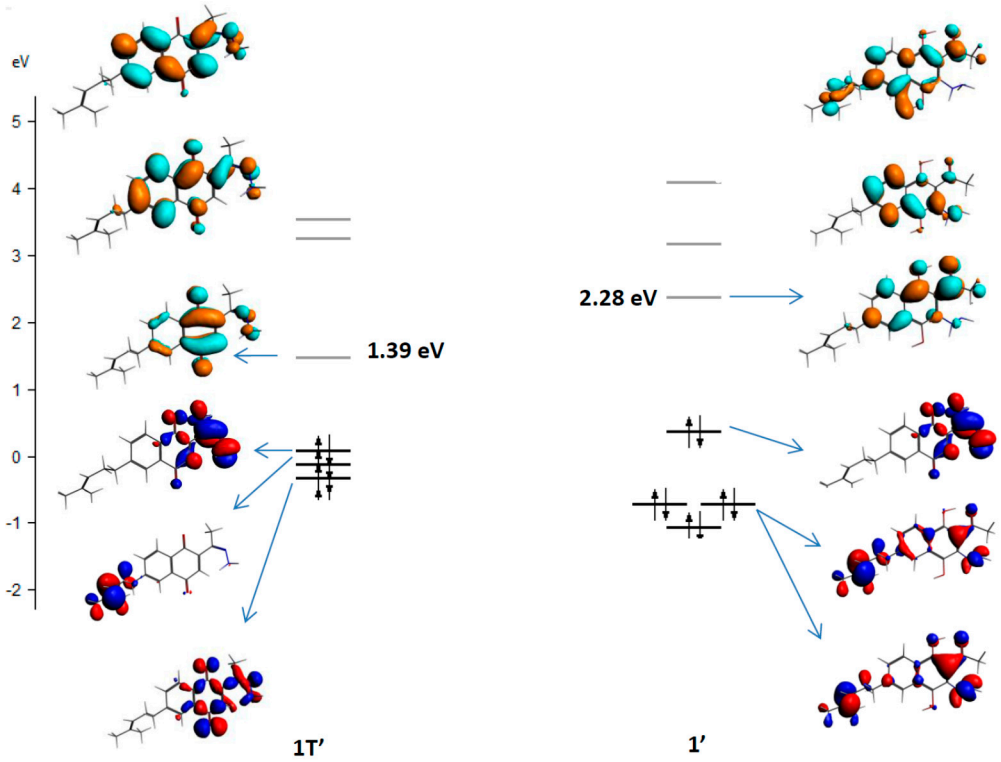
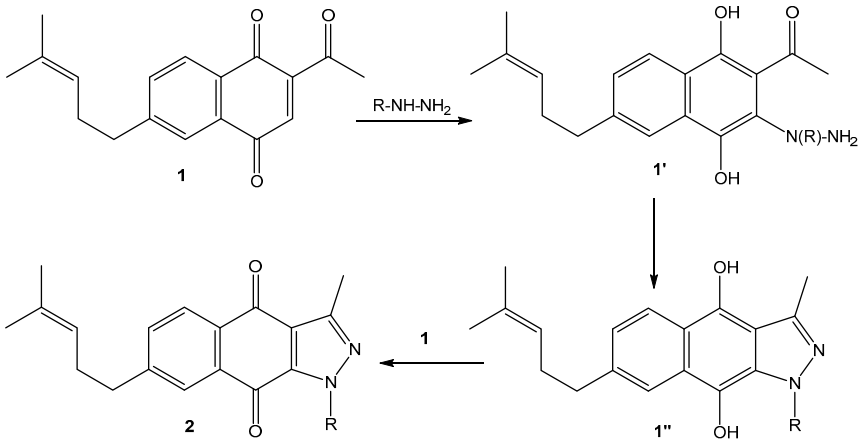


Figure 1. Molecular orbital diagrams of compounds **1T′** and **1′**. The HOMO energies have been arbitrarily set to zero for clarity.

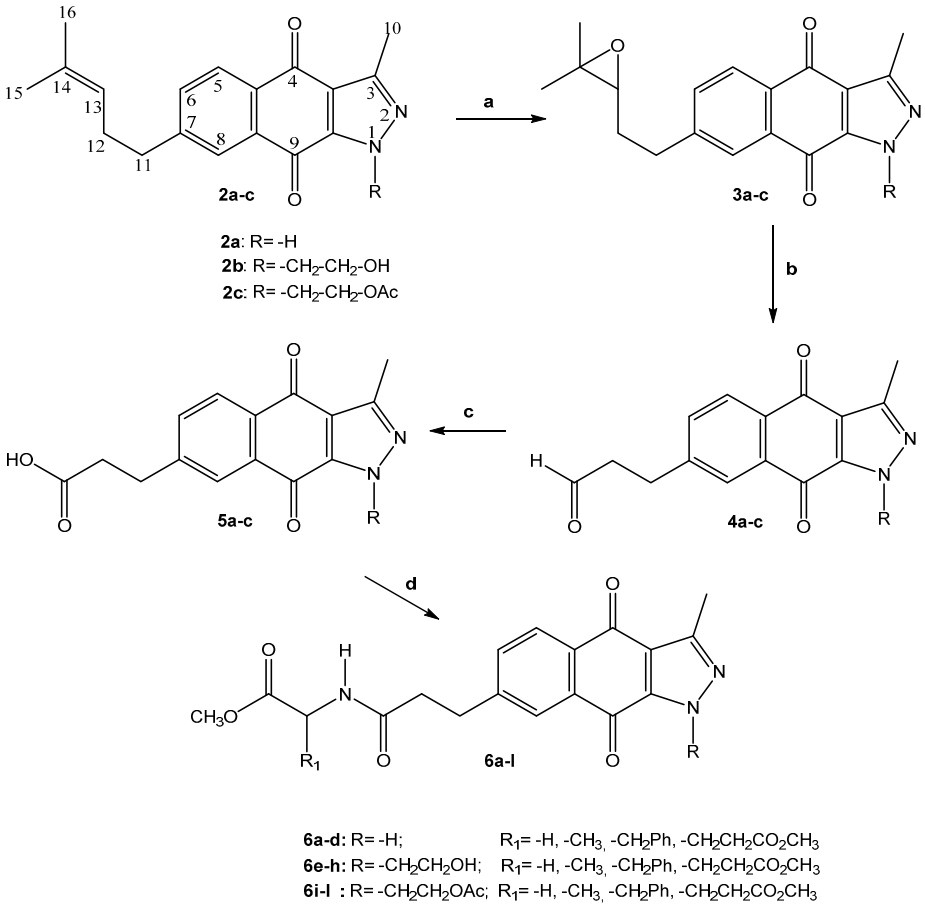
The second step of the reaction is the cyclization of the 3-substituted 1,4-hydroquinone compound **1′** by the nucleophilic addition/elimination reaction between the amino and carbonyl groups of **1′** to afford the fused pyrazolo-1,4-naphthohydroquinone compound **1″**. Finally, this compound is oxidized to the 1,4-naphthoquinone **2** by the initial 2-acetyl-1,4-naphthoquinone **1** (Scheme 2), a step that is supported by the isolation of 2-acetyl-6-(4-methylpent-3-enyl)-1,4-naphthohydroquinone as a by-product [10,28].



Scheme 2. Cyclization pathway for the *N*-substituted 1*H*-benzo[*f*]indazole-4,9-diones **2**.

To synthesize the new 1*H*-benzo[*f*]indazole-4,9-diones conjugated with C-protected amino acids **6a**–**l**, we followed the synthetic pathway shown in Scheme 3. The epoxidation of the double bond in the 7-(4-methylpent-3-enyl) group of **2a**–**c** to afford oxiranyl compounds **3a**–**c** was accomplished with *m*-chloroperoxybenzoic acid (mCPBA), and treatment of these compounds with periodic acid

afforded the aldehydes **4a–c** [29]. Oxidation of these aldehydes to the carboxylic acids **5a–c** was performed with sodium chlorite in the presence of a catalytic amount of 2-methyl-2-butene. The reactivity of the carboxylic group was then enhanced through the *in situ* formation of the mixed anhydride with ethyl chloroformate followed by the addition of the corresponding methyl ester of glycine, L-alanine, L-phenylalanine and L-glutamic acid [25]. In all of these synthesized compounds, the L-configuration must be retained in the amino acid unit. The physical and analytical data of the compounds are presented in the experimental section along with the IR, ^{1}H and ^{13}C spectroscopic data; chemical shifts are reported according to the carbon numbering of compounds **2** in Scheme 3.



Scheme 3. Synthetic pathway for the new conjugated derivatives **6a–l**. (**a**) mCPBA, CH_2Cl_2, $NaHCO_3$, rt, 4 h; (**b**) H_5IO_6, THF, H_2O, rt, 2 h; (**c**) $NaClO_2$, NaH_2PO_4, H_2O, *t*-BuOH, 2-methyl-2-butene, rt, 27 h; (**d**) EtOCOCl, Et_3N, THF, 20 min, 0 °C, $R_1CH(NH_2)CO_2Me$, rt, 16 h.

The common features from the spectral data of compounds **6a–l** are closely related to those previously reported for the starting compounds **2a–c** [23], and they are as follows:

- In some cases, their IR spectra show two carbonyl-quinone absorptions at approximately 1680 and 1670 cm^{-1}, but the latter absorption is primarily observed.
- In the ^{1}H spectra, the singlet of the C-10 methyl group appears at approximately 2.60 to 2.80 ppm, the coupled methylene groups of C-11 and C-12 carbons show triplets or multiplets between 2.50 and 3.00 ppm (J = 7.3–8.0 Hz), and the coupled aromatic hydrogen of carbon C-5, C-6 and C-8 are observed as doublets of doublets and two doublets at 7.70 to 8.10 ppm (J = 7.6 and 1.6 Hz).
- The ^{13}C-NMR spectra contain signals for carbonyl-quinone C-4 and C-9 carbon atoms at 170 to 180 ppm.

2.2. Biological Assay

The antiproliferative activity of the synthesized compounds was assessed on KATO-III and MCF-7 cell lines using a CellTiter 96® AQueous One Solution Proliferation Assay (MTS) from Promega (Madison, WI, USA) with doxorubicin as a control. The results were expressed as the concentration determining 50% inhibition of cell proliferation (IC_{50}).

Tables 1 and 2 show the IC_{50} values for the antiproliferative activity obtained for each derivative tested in KATO-III and MCF-7 cell lines, respectively. Each column in Tables 1 and 2 contains the IC_{50} values for derivatives belonging to Series-I, -II and -III, respectively.

The antiproliferative activity determined in KATO-III cell lines for Series-I, -II and -III of 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives ranged from 60.3 (**2a**) to 326.6 μM (**5a**), 25.5 (**2b**) to 401.8 μM (**3b**), and 33.0 (**2c**) to 324.3 (**3c**), respectively (Table 1). Similarly, the antiproliferative activity assayed in MCF-7 cell lines for Series-I, -II and -III 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives ranged from 63.2 (**2a**) to 432.5 μM (**3a**), 27.5 (**2b**) to 415.9 μM (**3b**), and 29.4 (**2c**) to 389.9 μM (**3c**), respectively (Table 2).

By comparing Tables 1 and 2 is possible to observe the similarity between the patterns generated from the IC_{50} values obtained from Series-I, -II and -III derivatives. Moreover, these patterns were very similar in both cell models.

Table 1. *In vitro* antiproliferative activities of 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives expressed as IC_{50} values obtained in KATO-III cell line.

Series-I **IC_{50} (CI 95%) μM** ***p***	**Series-II** **IC_{50} (CI 95%) μM** ***p***	**Series-III** **IC_{50} (CI 95%) μM** ***p***
2a 60.3 (18.9–192.4) C: NA, R: NA	**2b** 25.5 (9.4–69.1) C: NA, R: NS	**2a** 33.0 (8.0–136.5) C: NA, R: NS
3a 313.3 (110.4–889.3) C: ***, R: NA	**3b** 401.8 (166.8–967.8) C: ****, R: NS	**3c** 324.3 (132.9–791.3) C: ****, R: NS
4a 99.5 (50.1–197.7) C: ***, R: NA	**4b** 63.0 (22.4–176.9) C: NS, R: NS	**4c** 60.5 (29.8–123.1) C: NS, R: NS
5a 326.6 (167.9–635.4) C: ***, R: NA	**5b** 337.3 (192.3–591.5) C: ****, R: NS	**5c** 162.6 (70.6–374.2) C **, R: NS
6a 230.7 (82.9–642.2) C: **, R: NA	**6e** 310.5 (132.5–727.4) C: ****, R: NS	**6i** 208.0 (92.5–467.6) C: ***, R: NS
6b 114.8 (57.7–228.6) C: NS, R: NA	**6f** 43.5 (15.9–118.4) C: NS, R: *	**6j** 54.1 (13.6–215.1) C: NS, R: NS
6c 126.8 (41.3–389.4) C: NS, R: NA	**6g** 37.6 (11.5–123.4) C: NS, R: *	**6k** 34.9 (16.7–72.9) C: NS, R: **
6d 111.7 (35.3–353.1) C: NS, R: NA	**6h** 52.8 (16.8–166.7) C: NS, R: NS	**6l** 109.6 (32.5–370.4) C: NS, R: NS

The results are presented as means and 95% confidence intervals (CI 95%) for three independent experiments. C and R indicate column and row, respectively. *, **, *** and **** indicate significant differences at $p < 0.05$, 0.01, 0.001 and 0.0001, respectively. NA and NS indicate not available and not significant, respectively. Doxorubicin exhibited an IC_{50} of 4.0 μM (0.9–17.4) μM in KATO-III cell line.

Table 2. *In vitro* antiproliferative activities of 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives expressed as IC_{50} values obtained in MCF-7 cell line.

Series-I IC_{50} (CI 95%) μM *p*	Series-II IC_{50} (CI 95%) μM *p*	Series-III IC_{50} (CI 95%) μM *p*
2a 63.2 (24.8–161.5) C: NA, R: NA	**2b** 27.5 (71.1–106.8) C: NA, R: NS	**2c** 29.4 (14.1–61.3) C: NA, R: NS
3a 432.5 (167.8–1115.1) C: ****, R: NA	**3b** 415.9 (202.0–856.2) C: ****, R: NS	**3c** 389.9 (222.2–684.4) C: ****, R: NS
4a 123.6 (39.6–385.7) C: NS, R: NA	**4b** 43.4 (18.6–101.1) C: NS, R: *	**4c** 33.0 (10.8–100.7) C: NS, R: **
5a 372.4 (185.1–749.2) C: ***, R: NA	**5b** 335.0 (186.7–601.1) C: ****, R: NS	**5c** 244.9 (95.0–631.0) C: ****, R: NS
6a 413.0 (138.2–1234.7) C: ****, R: NA	**6e** 291.1 (155.1–546.2) C: ****, R: NS	**6i** 255.3 (115.8–562.8) C: ****, R: NS
6b 94.2 (44.8–197.9) C: NS, R: NA	**6f** 62.7 (17.0–231.2) C: NS, R: NS	**6j** 52.6 (24.2–114.2) C: NS, R: NS
6c 154.9 (59.2–405.2) C: NS, R: NA	**6g** 39.0 (11.6–131.1) C: NS, R: **	**6k** 35.4 (8.4–149.5) C: NS, R: ***
6d 143.9 (60.3–343.5) C: NS, R: NA	**6h** 87.9 (45.0–171.7) C: NS, R: NS	**6l** 99.8 (52.2–190.6) C: NS, R: NS

The results are presented as means and 95% confidence intervals (CI 95%) for three independent experiments. C and R indicate column and row, respectively. *, **, *** and **** indicate significant differences at $p < 0.05$, 0.01, 0.001 and 0.0001, respectively. NA and NS indicate not available and not significant, respectively. Doxorubicin exhibited an IC_{50} of 0.3 μM (0.3–1.3) μM in MCF-7 cell line.

A closer analysis using a two-way ANOVA test followed by a Dunnett's multiple comparison post-test showed that the most promising derivatives were compounds **2** (*i.e.*, **2a**, **2b** and **2c**), compounds **4** (*i.e.*, **4a**, **4b** and **4c**) and derivatives conjugated with L-alanine (*i.e.*, **6b**, **6f** and **6j**), L-phenylalanine (*i.e.*, **6c**, **6g** and **6k**) and L-glutamic acid (*i.e.*, **6d**, **6h** and **6l**). Additionally, the statistical analysis shows that the compounds of Series-II and -III have better IC_{50} values compared to compounds of Series-I.

3. Experimental Section

3.1. Chemistry

3.1.1. General

All reactions were performed using reagents and solvents purchased from commercial sources and purified by standard procedures as necessary. Starting *N*-substituted 1*H*-benzo[*f*]indazole-4,9-diones **2a**–**c** were synthesized according to a previously described procedure [23]. IR spectra were recorded on a Perkin Elmer FT IR 1600 spectrophotometer (Norwalk, CN, USA) as a film over NaCl discs. NMR spectra were recorded on a Bruker Avance 400 Digital NMR spectrometer (Bruker/Analytic, Karlsruhe, Germany) operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C in $CDCl_3$, acetone-d_6 or DMSO-d_6 with internal TMS as a reference. Chemical shifts (δ) were expressed in ppm, followed by multiplicity and coupling constant (*J*) in Hz. Elemental analyses of C, H and N were performed using a Perkin Elmer 2400 Series II CHN Elemental Analyzer (Perkin Elmer Inc., Waltham, MA 02451,

USA). The reaction progress was monitored by thin layer chromatography with Silica gel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) aluminum sheets, whereas column chromatographies were performed on Silica gel 60 (230–400 mesh, Merck) using solvent mixtures with variable proportions as eluents. Melting points were determined on a Stuart SMP 10 apparatus (Stone, Staffordshire, UK), and they were not corrected.

3.1.2. General Procedure for the Preparation of
7-[2-(3,3-Dimethyloxiranyl)-ethyl]-3-methyl-1*H*-benzo[*f*]indazole-4,9-diones **3a–c**

The compounds were synthesized by mCPBA (9.5 mmol) epoxidation of the 1*H*-benzo[*f*]indazole-4,9-dione **2a–c** (9.5 mmol) and 1.34 g of $NaHCO_3$ in CH_2Cl_2 (250 mL) at rt for 2 h under agitation. The crude epoxide was purified by column chromatography with *n*-hexane/ethyl acetate as the eluent.

7-[2-(3,3-Dimethyloxiranyl)-ethyl]-3-methyl-1H-benzo[f]indazole-4,9-dione (**3a**): This compound was prepared following the general procedure from 3-methyl-7-(4-methylpent-3-enyl)-1*H*-benzo[*f*]indazole-4,9-dione **2a**. Light orange solid purified with 1:1 hexane/ethyl acetate, 84% yield, m.p. 134–136 °C; IR (NaCl, ν/cm^{-1}) 3140 (NH), 1668 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.16 (s, 3H, CH_3, H16), 1.28 (s, 3H, CH_3, H15), 1.83–1.90 (m, 2H, CH_2, H12), 2.75 (s, 3H, CH_3, H10), 2.80 (t, *J* = 7.4 Hz, 1H, CH, H13), 2.87 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 7.97 (dd, J_1 = 7.9 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 8.06 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.15 (d, *J* = 7.9 Hz, 1H, CH, H5), 13.9 (s, 1H, NH, R). ^{13}C-NMR ($CDCl_3$) δ 11.7, 18.6, 24.7, 30.1, 32.8, 58.7, 63.5, 118.2, 127.0, 127.5, 128.2, 129.7, 130.2, 133.5, 134.0, 147.8, 178.3, 180.1. Elemental analysis calcd for $C_{18}H_{18}N_2O_3$: C 69;77; H 5.84; N 9.03; found: C 67.99; H 5.88; N 8.94.

7-[2-(3,3-Dimethyloxiranyl)-ethyl]-1-(2-hydroxyethyl)3-3-methyl-1H-benzo[f]indazole-4,9-dione (**3b**): This compound was prepared following the general procedure from 1-(2-hydroxy-ethyl)-3-methyl-7-(4-methylpent-3-enyl)-1*H*-benzo[*f*]indazole-4,9-dione **2b**. Brown solid purified with 1:1 hexane/ethyl acetate, 74% yield, m.p. 62–64 °C; IR (NaCl, ν/cm^{-1}) 3330 (OH), 1670, 1658 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.05 (s, 3H, CH_3, H16), 1.26 (s, 3H, CH_3, H15), 1.72–1.84 (m, 2H, CH_2, H12), 2.44 (s, 3H, CH_3, H10), 2.73 (t, *J* = 6.4 Hz, 1H, CH, H13), 2.84 (t, *J* = 6.4 Hz, 2H, CH_2, H11), 3.77 (t, *J* = 5.8 Hz, 2 H, CH_2N, R), 4.55 (t, *J* = 5.8 Hz, 2 H, CH_2O, R), 4.90 (s, 1H, OH, R), 7.67 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.86 (d, *J* = 1.6 Hz, 1H, CH, H8), 7.93 (d, *J* = 8.0 Hz, 1H, CH, H5). ^{13}C-NMR ($CDCl_3$) δ 13.0, 18.7, 24.8, 29.9, 32.3, 53.6, 58.1, 60.0, 62.7, 119.5, 126.7, 126.8, 132.0, 133.5, 134.8, 138.3, 148.1, 148.2, 175.7, 179.8. Elemental analysis calcd for $C_{20}H_{22}N_2O_4$: C 67.78; H 6.26; N 7.90; found: C 67.85; H 6.31; N 7.95.

2-{7-[2-(3,3-Dimethyloxiranyl)-ethyl]-3-methyl-4,9-dioxo-4,9-dihydro-benzo-[f]indazol-1-yl}-ethyl acetate (**3c**): This compound was prepared following the general procedure from 1-(2-acetoxyethyl)-3-methyl-7-(4-methylpent-3-enyl)-1*H*-benzo[*f*]indazole-4,9-dione **2c**. Yellow solid purified with 2:1 hexane/ethyl acetate, 64% yield, m.p. 104–106 °C; IR (NaCl, ν/cm^{-1}) 1744, 1669 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.50 (s, 3H, CH_3, H16), 1.63 (s, 3H, CH_3, H15), 1.98 (s, 3H, CH_3, R), 1.89–1.94 (m, 2 H, CH_2, H12), 2.61 (s, 3H, CH_3, H10), 2.77 (t, *J* = 6.2Hz, 1H, CH, H13), 2.92 (t, *J* = 6.5 Hz, 2H, CH_2, H11), 4.53 (t, *J* = 5.3 Hz, 2 H, CH_2N, R), 4.88 (t, *J* = 5.3 Hz, 2 H, CH_2O, R), 7.59 (dd, J_1 = 7.9 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 8.01 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.14 (d, *J* = 7.9 Hz, 1H, CH, H5). ^{13}C-NMR ($CDCl_3$) δ 13.1, 18.7, 20.7, 24.7, 30.2, 32.9, 50.3, 58.3, 62.3, 63.4, 120.0, 126.7, 127.3, 132.4, 133.4, 133.5, 134.5, 147.7, 149.6, 170.5, 176.3, 180.1. Elemental analysis calcd for $C_{22}H_{24}N_2O_5$: C 66.65; H 6.10; N 7.07; found: C 66.60; H 6.15; N 7.14.

3.1.3. General Procedure for the Preparation of *N*-Substituted
3-(3-Methyl-4,9-dioxo-4,9-dihydro-1*H*-benzo[*f*]indazol-7-yl)-propanal **4a–c**

These compounds were synthesized by the degradative oxidation of epoxides **3a–c** (0.31 mmol) dissolved in THF (10 mL) with H_5IO_6 (0.140 g, 0.61 mmol) in H_2O (3 mL) stirred 1 h at r.t.

After diluting with diethyl ether (20 mL), the organic phase was washed with a 5% aqueous solution of $Na_2S_2O_7$ (4 × 10 mL) and 5% Na_2CO_3 (10 mL). The products were purified by column chromatography with *n*-hexane/ethyl acetate as the eluent.

3-(3-Methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)-propanal (**4a**): This compound was prepared following the general procedure from epoxide **3a**. Yellow solid purified with 1:4 hexane/ethyl acetate, 72% yield, m.p. 248–250 °C; IR (NaCl, ν/cm^{-1}) 3198 (NH), 1720, 1666 (C=O). ^{1}H-NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3, H10), 2.88 (t, *J* = 7.2 Hz, 2H, CH_2, H12), 3.00 (t, *J* = 7.2 Hz, 2H, CH_2, H11), 7.69 (dd, J_1 = 7.8 Hz, J_2 = 1.6 Hz, 1 H, CH, H6), 7.98 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.10 (d, *J* = 7.8 Hz, 1H, CH, H5), 9.71 (s, 1H, H13), 13.7 (s, 1H, NH, R). ^{13}C-NMR (DMSO-d_6) δ 11.6, 28.6, 45.1, 116.6, 128.2, 129.9, 131.9, 133.6, 134.1, 142.3, 142.6, 145.1, 171.9, 180.5, 201.1. Elemental analysis calcd for $C_{15}H_{12}N_2O_3$: C 67.16; H 4.51; N 10.44; found: C 68.03; H 4.30; N 10.53.

3-[1-(2-Hydroxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)-propanal (**4b**): This compound was prepared following the general procedure from epoxide **3b**. Yellow orange solid purified with 1:1 hexane/ethyl acetate, 96% yield, m.p. 141–143 °C; IR (NaCl, ν/cm^{-1}) 3382 (OH), 1722, 1662 (C=O). ^{1}H-NMR ($CDCl_3$) δ 2.52 (s, 3H, CH_3 H10), 2.55 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 3.07 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 3.84 (t, *J* = 5.7 Hz, 2H, CH_2N, R), 4.64 (t, *J* = 5.7 Hz, 2H, CH_2O, R), 4.96 (s, 1H, OH, R), 7.75 (dd, J_1 = 7.8 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.98 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.01 (d, *J* = 7.8 Hz, 1H, CH, H5), 9.71 (s, 1H, H13). ^{13}C-NMR ($CDCl_3$) δ 13.1, 27.7, 44.4, 53.6, 60.0, 119.5, 126.7, 126.8, 132.1, 133.6, 134.8, 138.4, 146.8, 148.1, 175.4, 179.8, 202.4. Elemental analysis calcd for $C_{17}H_{16}N_2O_4$: C 65.38; H 5.16; N 8.97; found: C 65.41; H 5.19; N 9.00.

2-[3-Methyl-4,9-dioxo-7(3-oxopropyl)-4,9-dihydro-benzo[f]indazol-1-yl]-ethyl acetate (**4c**): This compound was prepared from epoxide **3c** following the general procedure. Yellow solid purified with 1:1 hexane/ethyl acetate, 98% yield, m.p. 90–91 °C; IR (NaCl, ν/cm^{-1}) 1732, 1718, 1666 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.97 (s, 3H, CH_3, R), 2.61 (s, 3H, CH_3, H10), 2.90 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 3.10 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 4.53 (t, *J* = 5.2 Hz, 2H, CH_2N, R), 4.87 (t, *J* = 5.2 Hz, 2H, CH_2O, R), 7.59 (dd, J_1 = 7.9 Hz, J_2 = 1.7 Hz, 1H, CH, H6), 7.99 (d, *J* = 7.9 Hz, 1H, CH, H8), 8.09 (d, *J* = 1.7 Hz, 1H, CH, H5), 9.80 (s, 1H, H13). ^{13}C-NMR ($CDCl_3$) δ 13.1, 20.7, 27.9, 44.4, 50.2, 62.2, 120.2, 126.5, 127.4, 132.6, 133.6, 133.8, 134.5, 146.7, 149.6, 175.6, 176.2, 180.0, 200.2. Elemental analysis calcd for $C_{19}H_{18}N_2O_5$: C 64.40; H 5.11; N 7.90; found: C 64.35; H 5.09; N 7.94.

3.1.4. General Procedure for the Preparation of *N*-Substituted 3-(3-Methyl-4,9-dioxo-4,9-dihydro-1*H*-benzo[*f*]indazol-7-yl)-propanoic Acids **5a–c**

These compounds were prepared via the oxidation of aldehydes **4a–c** (0.33 mmol) in *t*-BuOH (9 mL) with $NaClO_2$ (0.5 mL aqueous solution 25%), NaH_2PO_4 (0.4 mL aqueous solution 5%) and catalytic 2-methyl-2-butene (0.2 mL) at r.t. for 72 h. After acid work-up with 2 M HCl and extraction with ethyl acetate (3 × 10 mL) and CH_2Cl_2 (10 mL), the products were purified by column chromatography with hexane/ethyl acetate 2:1 as the eluent.

3-(3-Methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)-propanoic acid (**5a**): Following the general procedure, this compound was obtained from aldehyde **4a**. White solid, 98% yield, m.p. 288–290 °C; IR (NaCl, ν/cm^{-1}) 3410 (OH), 3204 (NH), 1704, 1667 (C=O). ^{1}H-NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3, H10), 2.62 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 2.98 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 7.71 (dd, J_1 = 8.2 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.93 (d, *J* = 1.6 Hz, 1H, CH, H8), 7.99 (d, *J* = 8.2 Hz, 1H, CH, H5), 12.23 (s, broad, 1H, H13), 14.20 (s, 1H, NH, R). ^{13}C-NMR (DMSO-d_6) δ 13.9, 30.1, 34.3, 114.1, 117.9, 126.5, 134.0, 134.2, 147.3, 173.4, 179.6. Elemental analysis calcd for $C_{15}H_{12}N_2O_4$: C 63.37; H 4.25; N 9.85; found: C 63.41; H 4.30; N 9.65.

3-[1-(2-Hydroxyethyl)3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoic acid (**5b**): Following the general procedure, this compound was obtained from aldehyde **4b**. Yellow solid, 64% yield, m.p. 218–219 °C; IR (NaCl, ν/cm^{-1}) 3331 (OH), 1700, 1664 (C=O). ^{1}H-NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3, H10), 2.63 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 2.98 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 3.77 (t, *J* = 5.5 Hz, 2H, CH_2N, R), 4.58 (t, *J* = 5.5 Hz, 2H, CH_2O, R), 4.90 (s, 1H, OH, R), 7.71 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H, CH, H6), 7.90 (d, *J* = 1.5 Hz, 1H, CH, H8), 8.00 (d, *J* = 1.5 Hz, 1H, CH, H5), 12.30 (s, broad, 1H, H13). ^{13}C-NMR (DMSO-d_6) δ 13.1, 30.5, 34.7, 53.6, 60.0, 119.5, 126.7, 126.8, 132.1, 133.6, 134.8, 147.8, 148.1, 173.5, 175.8, 179.9. Elemental analysis calcd for $C_{17}H_{16}N_2O_5$: C 62.19; H 4.91; N 8.53; found: C 62.14; H 4.86; N 8.48.

3-[1-(2-Acetoxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoic acid (**5c**): Following the general procedure, this compound was obtained from aldehyde **4c**. Grey solid, 48% yield, m.p. 186–187 °C; IR (NaCl, ν/cm^{-1}) 3400 (OH), 1742, 1705, 1670, 1664 (C=O). ^{1}H-NMR (DMSO-d_6) δ 1.90 (s, 3H, CH_3, R), 2.50 (s, 3H, CH_3, H10), 2.64, (t, *J* = 7.4 Hz, 2H, CH_2, H12), 3.00 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 4.45 (t, *J* = 5.2 Hz, 2H, CH_2N, R), 4.79 (t, *J* = 5.2 Hz, 2H, CH_2O, R), 7.74 (dd, J_1 = 7.9 Hz, J_2 = 1.4 Hz, 1H, H6), 7.97 (d, *J* = 1.4 Hz, 1H, CH, H8), 8.00 (d, *J* = 7.9 Hz, 1H, CH, H5), 12.05 (s, broad, 1H, H13). ^{13}C-NMR (DMSO-d_6) δ 12.7, 20.4, 30.1, 34.3, 49.8, 61.8, 119.3, 126.3, 126.5, 131.7, 133.1, 138.1, 148.0, 170.0, 173.4, 174.5, 179.5. Elemental analysis calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.96; N 7.50; Found: C 61.55; H 4.90; N 7.60.

3.1.5. General Procedure for the Preparation of [3-(3-Methyl-4,9-dioxo-4,9-dhydro-1*H*-benzo[*f*]indazol-7-yl)propanoylamino]-methyl Ester **6a–l**

A solution containing 0.37 mmol of carboxylic acids **5a–c**, 0.041 g (0.407 mmol, 56 μL) of triethylamine and 0.044 g (0.407 mmol, 38 μL) of ethyl chloroformate in 12 mL of dry THF was stirred for 20 min at 0 °C. After the addition of 0.407 mmol of the protected L-amino acid (Gly, Ala, Phe, and Glu), the mixture was stirred 16 h at r.t. After filtration over Celite-545 and evaporation of the solvent, the residue was dissolved in 70 mL of ethyl acetate. The organic solution was extracted with 40 mL of a 5% $NaHCO_3$ solution and water (40 mL). After drying with Na_2SO_4, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography with chloroform/acetone 7:3 as the eluent.

[3-(3-Methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)propanoylamino]-methyl acetate (**6a**): This compound was prepared following the general procedure from carboxylic acid **5a** and glycine methyl ester hydrochloride. White solid, 80% yield, m.p. 213–215 °C; IR (NaCl, ν/cm^{-1}) 3219 (NH), 1750, 1668, 1640 (C=O). ^{1}H-NMR (DMSO-d_6) δ 2.53 (t, *J* = 7.6 Hz, 2H, CH_2, H12), 2.57 (s, 3H, CH_3, H10), 2.99 (t, *J* = 7.6 Hz, 2H, CH_2, H11), 3.59 (s, 3H, CH_3O), 3.81 (d, *J* = 5.8 Hz, 2H, CH_2), 7.70 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H, CH, H6), 7.96 (d, *J* = 1.5 Hz, 1H, CH, H8), 7.99 (d, *J* = 7.9 Hz, 1H, CH, H5), 8.35 (d, *J* = 5.0 Hz, 1H, NH), 14.26 (s, 1H, NH, R). ^{13}C-NMR (DMSO-d_6) δ 11.0, 30.7, 35.7, 40.5, 51.6, 114.1, 117.8, 126.4, 126.5, 132.8, 134.0, 147.5, 170.8, 171.5, 179.6. Elemental analysis calcd for $C_{18}H_{17}N_3O_5$: C 60.84; H 4.82; N 11.82; found: C 60.85; H 4.87; N 11.90.

2-[3-(3-Methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)propanoylamino]-methyl propanoate (**6b**): This compound was prepared following the general procedure from carboxylic acid **5a** and L-alanine methyl ester hydrochloride. Yellow solid, 49% yield, m.p. 246–248 °C; IR (NaCl, ν/cm^{-1}) 3221 (NH), 1743, 1667, 1645 (C=O). ^{1}H-NMR (DMSO-d_6) δ 1.21 (d, *J* = 7.3 Hz, 3H, CH_3, R_1), 2.49 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 2.57 (s, 3H, CH_3, H10), 2.95 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 3.57 (s, 3H, CH_3O), 4.20 (q, *J* = 7.3 Hz, 1H, CH), 7.69 (dd, J_1 = 8.0 Hz; J_2 = 1.6 Hz, 1H, CH, H6), 7.96 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.01 (d, *J* = 8.0 Hz, 1H, CH, H5), 8.34 (d, *J* = 7.0 Hz, 1H, NH), 14.10 (s, 1H, NH, R). ^{13}C-NMR (DMSO-d_6) δ 10.8, 17.0, 30.7, 42.7, 51.7, 117.8, 126.3, 126.4, 126.6, 126.7, 134.0, 134.2, 147.3, 170.8, 173.0, 179.0. Elemental analysis calcd for $C_{19}H_{19}N_3O_5$: C 61.78; H 5.18; N 11.38; found: C 61.85; H 5.20; N 11.43.

2-[3-(3-Methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)propanoylamino]-methyl-3-phenyl propanoate (**6c**): This compound was prepared following the general procedure from carboxylic acid **5a** and L-phenylalanine methyl ester hydrochloride. Brown solid, 98% yield, m.p. 215–217 °C; IR (NaCl, ν/cm^{-1}) 3219 (NH), 1774, 1691, 1667 (C=O). ^{1}H-NMR ($CDCl_3$) δ 2.66 (t, *J* = 7.9 Hz, 2H, CH_2, H12), 2.85 (s, 3H, CH_3, H10), 3.06–3.12 (m, 4H, 2 CH_2, H11, R_1), 3.75 (s, 3H, CH_3O), 4.13 (q, *J* = 7.2 Hz, 1H, CH), 7.17–7.60 (m, 8H, CH, aromatic), 8.11 (s, broad, 1H, NH), 13.96 (s, 1H, NH, R). ^{13}C-NMR ($CDCl_3$) δ 14.1, 26.5, 37.5, 40.9, 51.9, 114.5, 126.2, 126.8, 127.1, 128.5, 129.2, 133.0, 137.1, 171.0 173.0, 175.3. Elemental analysis calcd for $C_{25}H_{23}N_3O_5$: C 67.41; H 5.20; N 9.40; found: C 67.35; H 5.25; N 9.60.

2-[3-(3-Methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)propanoylamino]-dimethyl pentane-dioate (**6d**): This compound was prepared following the general procedure from carboxylic acid **5a** and L-glutamic acid dimethyl ester hydrochloride. White solid, 38% yield, m.p. 222–224 °C; IR (NaCl, ν/cm^{-1}) 3226 (NH), 1746, 1691, 1669 (C=O). ^{1}H-NMR (DMSO-d_6) δ 1.70–1.90 (m, 2H, CH_2, R_1), 2.00–2.35 (m, 2H, CH_2, R_1), 2.52 (t, *J* = 7.3 Hz, 2H, CH_2, H12), 2.57 (s, 3H, CH_3, H10), 2.98 (t, *J* = 7.3 Hz, 2H, CH_2, H11), 3.46 (s, 3H, CH_3O), 3.57 (s, 3H, CH_3O), 4.10–4.30 (m, 1H, CH), 7.68 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H, CH, H6), 7.92 (d, *J* = 1.5 Hz, 1H, CH, H8), 7.99 (d, *J* = 7.9 Hz, 1H, CH, H5), 8.29 (d, *J* = 7.7 Hz, NH), 14.24 (s, 1H, NH, R). ^{13}C-NMR (DMSO-d_6) δ 11.0, 26.0, 29.3, 30.8, 35.7, 50.8, 51.2, 51.8, 114.1, 117.8, 126.4, 126.5, 132.7, 133.5, 133.9, 134.2, 147.4, 171.1, 172.0, 172.4, 179.6. Elemental analysis calcd for $C_{22}H_{23}N_3O_7$: C 59.85; H 5.25; N 9.52; found: C 60.01; H 5.56; N 9.63.

{3-[1-(2-Hydroxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoylamino}-methyl acetate (**6e**): This compound was prepared following the general procedure from carboxylic acid **5b** and glycine methyl ester hydrochloride. White solid, 80% yield, m.p. 188–190 °C; IR (NaCl, ν/cm^{-1}) 3325 (broad, NH, OH), 1762, 1742, 1671, 1651 (C=O). ^{1}H-NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3, H10), 2.53 (t, *J*= 7.5 Hz, 2H, CH_2, H12), 2.99 (t, *J* = 7.5 Hz, 2H, CH_2, H11), 3.60 (s, 3H, CH_3O), 3.77 (d, *J*= 5.6 Hz, 2H, CH_2), 3.81 (t, *J* = 5.6 Hz, 2H, CH_2, CH_2N, R), 4.61 (t, *J* = 5.6 Hz, 2H, CH_2O, R), 4.92 (s, 1H, OH, R), 7.71 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.93 (d, *J* = 1.6 Hz, 1H, CH, H8), 7.97 (*J* = 8.0 Hz, 1H, CH, H5), 8.38 (t, *J* = 5.6 Hz, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ 12.8, 30.4, 30.7, 35.7, 40.5, 51.6, 53.3, 59.6, 119.2, 124.9, 126.4, 126.5, 131.7 133.3, 134.5, 138.1, 147.8, 170.3, 171.5, 175.5, 179.6. Elemental analysis calcd for $C_{20}H_{21}N_3O_6$: C 60.15; H 5.30; N 10.52; found: C 60.19; H 5.34; N 10.53.

2-*{3-[1-(2-Hydroxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoylamino}-methyl propanoate* (**6f**): This compound was prepared following the general procedure from carboxylic acid **5b** and L-alanine methyl ester hydrochloride. Yellow solid, 49% yield, m.p. 181–182 °C; IR (NaCl, ν/cm^{-1}) 3317 (broad, NH, OH), 1729, 1663, 1647 (C=O). ^{1}H-NMR (DMSO-d_6) δ 1.22 (d, *J* = 7.2 Hz, 3H, CH_3, R_1); 2.50 (s, 3H, CH_3, H10), 2.52 (t, *J* = 7.2 Hz, 2H, CH_2, H12), 2.98 (t, *J* = 7.2 Hz, 2H, CH_2, H11), 3.59 (s, 3H, CH_3O), 3.79 (t, *J* = 5.6 Hz, 2H, CH_2N, R), 4.24–4.27 (m, 1H, CH), 4.61 (t, *J* = 5.6 Hz, 2H, CH_2O, R), 4.92 (s, 1H, OH), 7.70 (dd, J_1 = 7.8, J_2 = 1.5 Hz, 1H, CH, H6), 7.93 (d, *J*= 1.5 Hz, 1H, CH, H8), 7.97 (d, *J* = 7.8 Hz, 1H, CH, H5), 8.35 (d, *J* = 7.2 Hz, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ 12.8, 17.0, 30.7, 35.7, 47.4, 51.7, 53.2, 59.6, 119.1, 126.4, 131.7, 133.2, 134.5, 138.1, 147.7, 170.8, 173.1, 175.5, 179.6. Elemental analysis calcd for $C_{21}H_{23}N_3O_6$: C 61.01; H 5.61; N 10.16; found: C 61.05; H 5.66; N 10.21.

2-{3-[1-(2-Hydroxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoylamino}-methyl-3-phenylpropanoate (**6g**): This compound was prepared following the general procedure from carboxylic acid **5b** and L-phenylalanine methyl ester hydrochloride. Brown solid, 98% yield, m.p. 154–156 °C. IR (NaCl, ν/cm^{-1}) 3309 (broad, NH, OH), 1743, 1659, 1644 (C=O). ^{1}H-NMR (DMSO-d_6) δ 2.44 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 2.48 (s, 3H, CH_3, H10), 2.75–3.00 (m, 4H, CH_2, CH_2, H11, R_1), 3.56 (s, 3H, CH_3O); 3.77 (t, *J* = 5.6 Hz, 2H, CH_2N, R), 4.43–4.45 (m, 1H, CH) 4.61 (t, *J* = 5.6 Hz, 2H, CH_2O, R), 4.90 (s,1H, OH), 7.12–7.22 (m, 5H, CH, aromatic, R_1), 7.60 (dd, J_1 = 7.9 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.80 (d, *J* = 1.6 Hz, 1H, CH, H8), 7.94 (d, *J* = 7.9 Hz, 1H, CH, H5), 8.39 (d, *J* = 7.8 Hz,

1H, NH). ^{13}C-NMR (DMSO-d_6) δ 12.8, 30.7, 35.7, 36.7, 51.8, 53.3, 53.4, 59.6, 119.2, 126.4, 128.1, 128.9, 131.7, 133.2, 134.4, 137.1, 138.1, 147.7, 147.8, 171.0, 172.0, 175.5, 179.6. Elemental analysis calcd for $C_{27}H_{27}N_3O_6$: C 66.25; H 5.56; N 8.58; found: C 66.21; H 5.50; N 7.92.

2-{3-[1-(2-Hydroxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)propanoylamino]-dimethyl pentanedioate (**6h**): This compound was prepared following the general procedure from carboxylic acid **5b** and L-glutamic acid dimethyl ester hydrochloride. Yellow solid, 44% yield, m.p. 160–162 °C. IR (NaCl, ν/cm^{-1}) 3306 (broad, NH, OH), 1738, 1675, 1644 (C=O). ^{1}H-NMR (DMSO-d_6) δ 1.60–2.00 (m, 2H, CH_2, R_1), 2.12–2.19 (m, 2H, CH_2, R_1), 2.49 (s, 3H, CH_3, H10), 2.52 (t, *J* = 7.6 Hz, 2H, CH_2, H12), 2.96 (t, *J* = 7.6 Hz, 2H, CH_2, H11), 3.47 (s, 3H, CH_3O), 3.58 (s, 3H, CH_3O), 3.78 (t, *J* = 5.7 Hz, 2H, CH_2N, R), 4.23–4.25 (m, 1H, CH), 4.60 (t, *J* = 5.7 Hz, 2H, CH_2O, R), 4.91(s,1H, OH), 7.68 (dd, J_1 = 7.1 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.92 (d, *J* = 1.6 Hz, 1H, CH, H8), 7.97 (d, *J* = 7.1 Hz, 1H, CH, H5), 8.28 (d, *J* = 7.7 Hz, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ 12.8, 26.0, 29.4, 30.8, 35.7, 50.9, 51.2, 51.8, 53.2, 59.6, 119.1, 126.4, 131.7, 133.2, 134.5, 138.1, 147.6, 147.7, 171.1, 172.0, 172.4, 175.5, 179.5. Elemental analysis calcd for $C_{24}H_{27}N_3O_8$: C 59.37; H 5.61; N 8.66; found: C 59.42; H 5.70; N 8.71.

{3-[1-(2-Acetoxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoylamino}-methyl acetate (**6i**): This compound was prepared following the general procedure from carboxylic acid **5c** and glycine methyl ester hydrochloride. Yellow oil, 41% yield. IR (NaCl, ν/cm^{-1}) 3330 (broad, NH), 1742, 1670, 1664, 1658 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.98 (s, 3H, CH_3, R), 2.61 (s, 3H, CH_3, H10), 2.65 (t, *J* = 7.4 Hz, 2H, CH_2, H12), 3.14 (t, *J* = 7.4 Hz, 2H, CH_2, H11), 3.75 (s, 3H, CH_3O), 4.01 (d, *J* = 5.0 Hz, 2H, CH_2), 4.53 (t, *J* = 5.2 Hz, 2H, CH_2N, R), 4.87 (t, *J* = 5.2 Hz, 2H, CH_2O, R), 6.06 (d, *J* = 5.0 Hz, 1H, NH), 7.62 (dd, J_1 = 7.9 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 8.00 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.12 (d, *J* = 7.9 Hz, 1H, CH, H5). ^{13}C-NMR ($CDCl_3$) δ 13.5, 21.1, 30.1, 31.6, 31.7, 37.1, 37.2, 41.7, 50.7, 52.8, 62.7, 120.7, 127.0, 127.8, 133.0, 134.1, 135.0, 138.7, 147.5, 148.8, 150.0, 170.3, 171.0, 171.1, 176.7, 180.5. Elemental analysis calcd for $C_{22}H_{23}N_3O_7$: C 59.85; H 5.25; N 9.52; found: C 59.91; H 5.32; N 9.85.

2-{*3-[1-(2-Acetoxyethy)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoylamino}-methyl propanoate* (**6j**): This compound was prepared following the general procedure from carboxylic acid **5c** and L-alanine methyl ester hydrochloride. Amber solid, 56% yield, m.p. 130–132 °C. IR (NaCl, ν/cm^{-1}) 3312 (broad, NH), 1744, 1670, 1654, 1648 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.31 (d, *J* = 7.1 Hz, 3H, CH_3, R_1), 1.91 (s, 3H, CH_3, R), 2.50 (s, 3H, CH_3, H10), 2.52 (t, *J* = 7.7 Hz, 2H, CH_2, H12), 3.06 (t, *J* = 7.7 Hz, 2H, CH_2, H11), 3.67 (s, 3H, CH_3O,), 4.45 (t, *J* = 5.2 Hz, 2H, CH_2N, R), 4.49 (q, *J* = 7.1 Hz, 1H, CH), 4.80 (t, *J* = 5.2 Hz, 2H, CH_2O, R), 5.99 (d, *J* = 7.1 Hz, 1H, NH), 7.54 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H, CH, H6), 7.93 (d, *J* = 1.5 Hz, 1H, CH, H8), 8.06 (d, *J* = 7.9 Hz, 1H, CH, H5). ^{13}C-NMR ($CDCl_3$) δ 13.5, 18.9, 21.1, 30.1, 31.6, 37.4, 48.4, 49.0, 50.7, 52.9, 125.5, 127.0, 127.8, 130.3, 132.2, 134.0, 135.0, 147.5, 150.0, 171.0, 171.5, 173.9, 176.7, 180.9. Elemental analysis calcd for $C_{23}H_{25}N_3O_7$: C 60.65; H 5.53; N 9.23; found: C 60.50; H 5.60; N 9.30.

2-{3-[1-(2-Acetoxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl]-propanoylamino}-methyl 3-phenylpropanoate (**6k**): This compound was prepared following the general procedure from carboxylic acid **5c** and L-phenylalanine methyl ester hydrochloride. Yellow oil, 69% yield. IR (NaCl, ν/cm^{-1}) 3302 (broad, NH), 1744, 1668, 1648 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.97 (s, 3H, CH_3, R), 2.57 (t, *J* = 7.5 Hz, 2H, CH_2, H12), 2.62 (s, 3H, CH_3, H10), 3.07–3.10 (m, 4H, CH_2, CH_2, H11, R_1), 3.72 (s, 3H, CH_3O), 4.52 (t, *J* = 5.3 Hz, 2H, CH_2N, R), 4.86–4.88 (m, 3H, CH, CH_2O, R), 5.88 (d, *J* = 7.6 Hz, 1H, NH), 7.23–7.27 (m, 5H, aromatic CH, R_1), 7.58 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, CH, H6), 7.99 (d, *J* = 1.6 Hz, 1H, CH, H8), 8.12 (d, *J* = 8.0 Hz, 1H, CH, H5). ^{13}C-NMR ($CDCl_3$) δ 13.5, 21.1, 31.5, 37.4, 38.2, 50.7, 52.8, 53.4, 120.2, 127.0, 127.6, 127.8, 129.0, 129.6, 134.0, 135.0, 136.0, 147.4, 150.0, 171.0, 171.1, 172.3, 180.2. Elemental analysis calcd for $C_{29}H_{29}N_3O_7$: C 65.53; H 5.50; N 7.90; found: C 65.48; H 5.60; N 7.96.

2-{3-[1-(2-Acetoxyethyl)-3-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indazol-7-yl)propanoylamino]-dimethyl pentanedioate (**6l**): This compound was prepared following the general procedure from carboxylic acid **5c** and L-glutamic acid dimethyl ester hydrochloride. Yellow oil, 48% yield. IR (NaCl, ν/cm^{-1}) 3330 (broad, NH), 1740, 1670, 1644 (C=O). ^{1}H-NMR ($CDCl_3$) δ 1.99 (s, 3H, CH_3, R), 2.10–2.40 (m, 4H, CH_2, CH_2, R_1), 2.60 (s, 3H, CH_3, H10), 2.62 (t, J = 7.8 Hz, 2H, CH_2, H12), 3.12 (t, J = 7.8 Hz, 2H, CH_2, H11), 3.63 (s, 3H, CH_3O), 3.76 (s, 3H, CH_3O), 4.53 (t, J = 5.2 Hz, 2H, CH_2N, R), 4.60 (d, J = 5.0 Hz, 1H, CH), 4.80 (t, J = 5.2 Hz, 2H, CH_2O, R), 6.26 (d, J = 7.4 Hz, 1H, NH), 7.62 (dd, J_1 = 7.9 Hz, J_2 = 1.7 Hz, 1H, CH, H6), 8.01 (d, J = 1.7 Hz, 1H, CH, H8), 8.12 (d, J = 7.9 Hz, 1H, CH, H5). ^{13}C-NMR ($CDCl_3$) δ 13.5, 21.1, 27.6, 30.3, 31.6, 37.4, 50.7, 52.1, 52.3, 53.0, 122.0, 127.0, 127.8, 134.1, 135.0, 138.7, 147.4, 150.1, 171.0, 171.5, 172.6, 173.7, 175.0, 180.1. Elemental analysis calcd for $C_{26}H_{29}N_3O_9$: C 59.20; H 5.54; N 7.97; found: C 59.13; H 5.72; N 8.01.

3.2. Computational Details

DFT calculations [30–33] were conducted using the Amsterdam Density Functional (ADF) program [34]. The Vosko-Wilk-Nusair parametrization [35] was used to treat electron correlation within the local density approximation (LDA). The numerical integration procedure applied for the calculation was developed by teVelde [33]. The standard ADF TZ2P basis set was used for all atoms. The frozen core approximation was used to treat core electrons at the following levels: C, 1s; N, 1s; and O, 1s [33]. Full geometry optimizations were performed on each complex using the analytical gradient method implemented by Verluis and Ziegler [36]. The geometries for all the model compounds discussed in the text were fully optimized and checked via analytical frequency calculations as either true minima (no imaginary values).

3.3. Antiproliferative Assay

KATO-III (human gastric cancer cell line) and MCF-7 (human breast adenocarcinoma cell line) cells were obtained from the American Type Culture Collection (ATCC). KATO-III and MCF-7 cells (2×10^3) were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were subcultured into fresh medium (100-mm-diameter plate dish) until a density of approximately 80% was obtained. Briefly, 2×10^3 cells were seeded in 96-well culture plates. After 24 h of incubation at 37 °C in a humidified 5% CO_2 atmosphere, different concentrations (10^{-9} to 10^{-3} M) of 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives were added. After 72 h of incubation, 20 µL of MTS (Promega, Madison, WI, USA) was added, and the wells were incubated for an additional 2 h at 37 °C. The absorbance at 490 nm was recorded using a Varioskan Flash Multimode Reader (Thermo Scientific, Waltham, MA, USA). Each variant of the experiment was performed in triplicate. To obtain IC_{50} values for each compound, dose-response curves were constructed in both KATO-III and MCF-7 cell lines. Doxorubicin was included in all evaluation to provide a reference of antiproliferative activity.

3.4. Statistical Analysis

Data are expressed as means ± CI 95% (95% confidence intervals) for three independent experiments. The concentration inducing a 50% decrease of cell proliferation (IC_{50}) was performed using the four-parameters logistic fit—also known as "4PL"—supported by GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Statistical differences among means were assessed using a two-way ANOVA test followed by a Dunnett's multiple comparison post-test. A $p < 0.05$ was taken as statistically significant.

4. Conclusions

In this study, we have synthesized three series of new 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives containing oxiranyl, formyl, carboxylic and L- and *C*-protected *N*-aminoacidyl substituents attached to the side chain of the 1,4-naphthoquinone group in moderate to good yields.

All compounds were characterized using spectroscopic techniques, namely, FT-IR, ^{1}H-NMR, and ^{13}C-NMR, and their data are in agreement with their proposed structures. DFT calculations provide the first insights into the reaction pathways; further investigations to clarify the entire reaction pathway are currently in progress. These families of compounds contain, in a single structure, a 1,4-quinone group fused to a pyrazolyl heterocyclic ring, substituents that are present individually in anticancer drugs such as doxorubicin, daunorubicin or in heterocyclic compounds with antitumoral properties. Moreover, they contain an amino acid group capable of orienting their transport into the cell organelles, where they could interfere with protein synthesis. Preliminary antiproliferative activity analyses showed that most of the derivatives presented some degree of activity. However, the derivatives **2**, **4** and those conjugated with L-alanine, L-phenylalanine and L-glutamic acid, and especially those belonging to Series-II and -III, presented the highest activity, as indicated by their IC_{50} values. These results suggest that 1*H*-benzo[*f*]indazole-4,9-dione-based derivatives are promising compounds for the development of anticancer drugs.

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Sample Availability: Samples of the compounds **2a**–**c**–**6a**–**l** are not available from the authors.