*Research Article*

# Synthesis of 4,7,9-Trihydroxy[1]benzofuro[3,2-d] pyrimidine-6-carboxamide: Evaluation of Cytotoxicity and Inhibition of Protein Kinase C (*Ca*Pkc1)

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The protein kinase Pkc1 of *Candida albicans* (*Ca*Pkc1), one of the key proteins involved in MAPK pathway, is described as a regulator of cell wall integrity during growth, morphogenesis, and response to cell wall stress. The (–)-cercosporamide is an antifungal natural product isolated from the phytopathogen fungus *Cercosporidium henningsii*. This phytoxin was found to inhibit selectively *Ca*Pkc1 and constitutes an interesting model for the design of novel antifungal molecules. In this research, 4,7,9-trihydroxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (**13**) derived from (–)-cercosporamide was synthesized via a seven-step procedure by well-known reactions and evaluation of cytotoxicity and inhibition of *Ca*Pkc1. The bioassay showed CaPkc1 inhibitory activity 87% higher and cytotoxicity 100 times less than the reference, (–)-cercosporamide.

## 1. Introduction

Candidiasis exists in all regions of the world; however, there are significant variations between regions and it is difficult to give an average incidence of candidiasis. Although many studies have been carried out annually, the type of population recruited is variable and the results rendered are difficult to compare. In addition, it is absolutely necessary to know the profile of the local epidemiology in order to best adapt the antifungal treatment. American studies report that the annual incidence of *Candida* infections is 8 per 100,000 inhabitants [1, 2]. In Europe, *Candida* spp. are responsible for 2-3% of septicemia ranking between 6th and 10th among pathogens most frequent [3]. The distribution of *Candida* species in patients with candidiasis has changed over the past 20 years. The incidence of *Candida albicans* decreased (from 70% to 50%), while the incidence of non-*albicans Candida* (NAC) species, including *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. auris*, and *C. parapsilosis* has increased [4]. Until recently, *C. albicans* remains mainly responsible for these invasive infections, but the distribution of NAC species evolves and differs according to the regions of the world [5]. In *C. albicans*, protein kinase C (*Ca*Pkc1) is one of the proteins involved in the MAPK pathway [6]. It is described as a regulator of the integrity of the cell wall during growth, morphogenesis, and the response to cell wall stress [7]. Treatment of fungal infections is very difficult because of limited therapeutic arsenal and emergence of resistance to the two antifungal major classes: azoles and echinocandins. Consequently, the search for new targets and new therapeutic strategies is a priority. In this context, targeting PKC-mediated signal transduction pathway represents a new attractive strategy for antifungal therapy. The (–)-cercosporamide is an antifungal natural product isolated from the phytopathogen fungus *Cercosporidium henningsii* [8]. This phytoxin was found to inhibit selectively Pkc1 kinase of *C. albicans* ($IC_{50} = 44$ nM) [9] and constitutes an interesting model for the design of novel antifungal molecules. In addition, research results suggest dihydroxybenzofuran-carboxamide ring as the pharmacophore of the molecule,

justifying the strategy for the design of new tricyclic compounds [10]. Regarding this, our expertise in the synthesis of pyrimidine-fused heterocycles as biological agents targeting kinases was used for the design of the new compounds, constituting the third ring of the benzofuro[3,2-d]pyrimidine derivatives described [11]. In this study, we designed and synthesized a new compound **13,** which remained the dihydroxybenzofuran-carboxamide ring (part A, excluding asymmetric carbon), replacing part B with pyrimidine ring (Figure 1) targeting *Ca*Pkc1. The introduction of a pyrimidine ring into the structure is capable of providing donor or acceptor groups for hydrogen bonds and can cause additional interactions with amino acids at the active site for better kinase inhibitory activity and improved selectivity.

## 2. Results and Discussion

*2.1. Chemistry.* As shown in Figure 2, analogues of (−)-cercosporamide were prepared from 3,5-dimethoxyphenol (**1**). Diiodination of compound **1** was performed very quickly at low temperature in the presence of NIS, followed by O-alkylation with a base and ethyl bromoacetate [12, 13] to afford **3** in 72%. Subsequent cyanation of this intermediate in the presence of cuprous cyanide in DMF gave ester **4** in 75% [14]. Cyclization using sodium hydride furnished benzofuran ring bearing the amino group at position 3 and ester function at position 2 [15]. Tricyclic pyrimidine derivative **6** was synthesized from benzofuran precursor **5** [16]. From compound **6**, attempts for the conversion of nitrile to carboxamide remained unsuccessful [13, 17–19].

Another strategy was to introduce the carboxamide group before the cyclization step. As shown in Figure 3, a direct aminocarbonylation of compound **1** use chlorosulfonylisocyanate (CSI) as electrophilic reagent afforded benzamide derivative **8** after acidic hydrolysis of the corresponding *N*-chlorosulfonyl carboxamide intermediate [20]. Next, the iodination of compound **8** was performed very quickly at 0°C in the presence of NIS, followed by cyanation in the presence of cuprous cyanide in DMF, and then, O-alkylation with a base and ethyl bromoacetate gave ester **11** in 90%. Afterwards, the same reaction sequence as previously described in Scheme 1 was applied to obtain derivative **7** bearing the carbamoyl appendage at C-6 position of the azaheterocycle. Finally, demethylation of the methoxy groups was carried out by heating in neat pyridine hydrochloride at 200°C under microwave irradiation to provide the desired 4,7,9-trihydroxy[1]benzofuro[3, 2-d] pyrimidine-6-carboxamide (**13**) in 43% [21]. The structures of all compounds synthesized were confirmed using MS, $^{1}$H, and $^{13}$C NMR spectrum (Supplementary Materials).

*2.2. Biological Activity.* The inhibition of PKC activity (*Ca*Pkc1) was initially investigated in order to check the involvement of this protein as the putative target of 4, 7, 9-trihydroxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (**13**). Pkc activity of *C. albicans* (CAAL93 sensitive to fluconazole); total protein extracts was measured using ELISA-
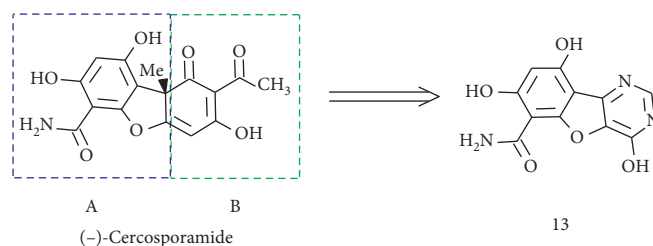


Figure 1: Design of target compound **13**.

based PKC Kinase Activity Assay (Enzo Life Sciences, Inc.) [22]. PKC inhibition activity was expressed as the ratio between the absorbance measured for protein extract treated with compounds **13** (100 $\mu$M) and untreated protein extract. The reference natural (−)-cercosporamide was provided by extraction and purification from a *Mycosphaerella henningsii* culture using a modified procedure of the literature [8, 23].

Mean percentage of inhibition of PKC activity was obtained for each compound (100 $\mu$M) and compared with mean results obtained for (−)-cercosporamide (100 $\mu$M) applying a one-way ANOVA followed by multiple comparisons with an uncorrected Fisher's LSD test. *P* values were expressed for the uncorrected Fisher's LSD test ($p < 0.00001$). To investigate in vitro cytotoxic activity of 4,7,9-trihydroxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (13), assay on HeLa cells was performed. As given in Table 1, compound **13** proved to be at least 100 times less cytotoxic than (−)-cercosporamide. This shows that compound **13** has the potential to become a new selective effect on fungal cells, not toxic to other cells. As depicted in Table 1, when used at 100 $\mu$M, (−)-cercosporamide displayed a moderate Pkc inhibitory activity of 53 ± 3% in our conditions. Interestingly, compound **13** showed statistically ($p < 0.05$) significant higher inhibition with values of 87 ± 2% at 100 $\mu$M. It is possible that the pyrimidine ring in part B in the structure has an additional –OH group resulting in stronger binding capacity of compound **13** to the center associated with *Ca*Pkc1, increasing the ability to inhibit *Ca*Pkc1 of compound 13.

## 3. Conclusions

An efficient and practically feasible procedure for the synthesis of 4,7,9-trihydroxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide via a seven-step procedure using common chemical reactions such as direct aminocarbonylation, iodonation, cyanation, O-alkylation, cyclizaction, and demethylation has been described. In this research, the initial biological assay of compound **13** suggests further chemical modifications to evaluate the structure-activity relationship of this naturally derived potential compound, (−)-cercosporamide.

## 4. Experimental

All reactions were monitored by TLC analysis using Merck silicagel 60F-254 thin-layer plates. Column chromatography was carried out on silicagel Merck 60 (70–230 mesh ASTM).
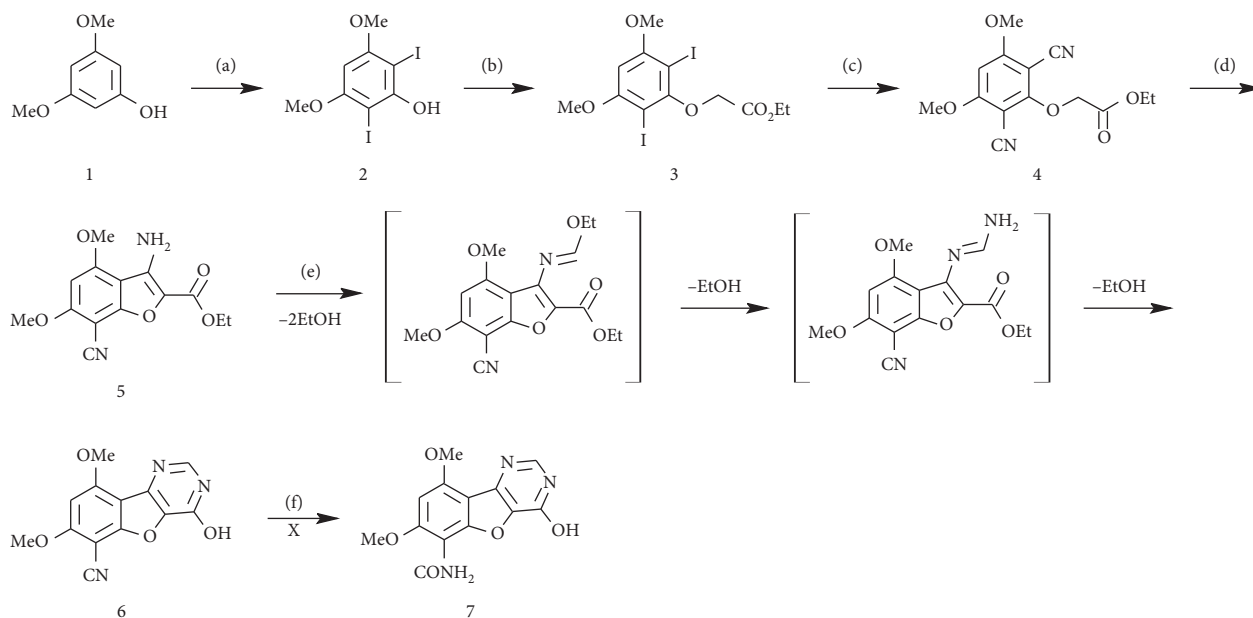
FIGURE 2: (a) NIS, CH$_2$Cl$_2$, −78°C, 2 min, 71%. (b) NaH 60%, DMF, ethyl bromoacetate, rt, 12 h, 72%. (c) CuCN, DMF, 160°C, 2 h, 75%. (d) NaH 60%, DMF, 0°C, 30 min, 51%. (e) (1) HC (OEt)$_3$, MW, 200°C, 15 min, (2) NH$_3$/MeOH 7N, MW, 140°C, 15 min, 46%. (f) H$_2$O$_2$ 35%, K$_2$CO$_3$, rt, 24 h or NaOH/EtOH 6N, H$_2$O$_2$ 35%, rt, 24 h or urea-hydrogen peroxide, K$_2$CO$_3$, acetone/H$_2$O, rt, 5 h or BF$_3$.2CH$_3$COOH or H$_2$SO$_4$ cc, 15 min, failure.
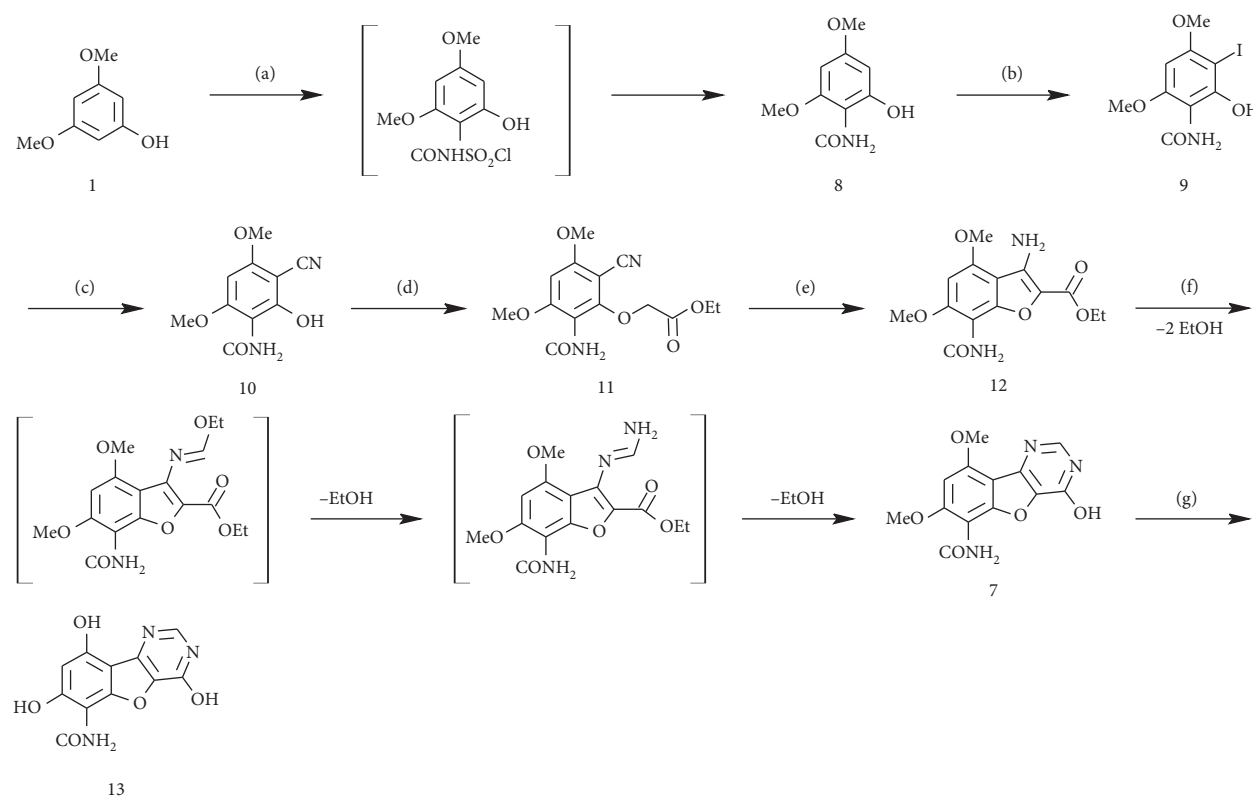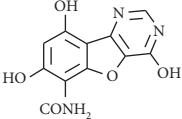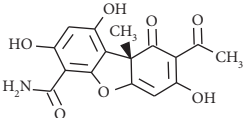


FIGURE 3: (a) CSI, CH$_3$CN, 0°C, 10 min and then HCl 5 M, rt, 10 h, 45%. (b) NIS, CH$_2$Cl$_2$, 0°C, 10 min, 60%. (c) CuCN, DMF, 160°C, 6 h, 38%. (d) NaH, BrCH$_2$CO$_2$Et, DMF, rt, 24 h, 90%. (e) NaH, DMF, 0°C, 10 min, 67%. (f) HC (OEt)$_3$, MW, 200°C, 15 min and then NH$_3$/MeOH 7N, MW, 140°C, 15 min, 62%. (g) Pyridine. HCl, MW, 200°C, 10 min, 43%.

Melting points were determined on an Electrothermal IA 9000 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Shimadzu IRAffinity-1 IR-FT spectrophotometer equipped with a MIRacle 10 accessory ATR. $^1$H and $^{13}$C NMR spectra were performed in DMSO-d$_6$ or CDCl$_3$ using a Bruker Avance 400 MHz spectrometer.

Table 1: Activity of compound **13** on protein extract of CAAL93 and HeLa.

| Compounds | Structure | MIC values ($\mu$M)[a] | |
| --- | --- | --- | --- |
| | | HeLa | Protein extract of CAAL93 |
| **13** |  | $227.53 \pm 27.97$ | $87 \pm 2\%$ |
| CERCO[b] |  | <3 | $53 \pm 3\%$ |

[a]Values represent the mean $\pm$ SD of experiments performed in triplicate. MIC was determined as the compound concentration that produced 50% of growth inhibition relative to that of the drug-free growth control. [b]CERCO, (–)-cercosporamide.

Chemical shifts are reported as $\delta$ values in parts per million (ppm) relative to tetramethylsilane as internal standard, and coupling constants (J) are given in Hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were recorded using an electrospray ionization method with the Waters ZQ 2000 spectrometer. Microwave-promoted reactions were performed on a CEM Discover SP monomode apparatus. Elemental analyses were performed on a Thermo Scientific Elemental Analyser Flash EA 1112 and were found within $\pm 0.4\%$ of the theoretical values.

*4.1. 2,6-Diiodo-3,5-dimethoxyphenol (2).* A solution of 3,5-dimethoxyphenol (**1**) (1.00 g, 6.50 mmol) in dichloromethane (20 mL) was cooled to −78°C. N-Iodosuccinimide (3.22 g, 14.3 mmol) was added, and the reaction was maintained at −78°C for 2 min. The reaction was quenched at −78°C by the addition of 10% aqueous potassium carbonate solution (20 mL) and warmed to room temperature. The reaction was diluted with water (50 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography using cyclohexane/ethyl acetate (9/1) as an eluent system to give compound **2** as a beige solid (1.87 g, 71%). $R_f = 0.32$ (cyclohexane/ethyl acetate: 8/2); Mp = 112–113°C. IR (cm$^{-1}$): 3423, 1496, 1446, 1379, 1336, 1292, 1184, 1093, 1064, 1033, 804, 729, 692. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 6.05 (s, 1H, H-4), 5.94 (s, 1H, OH), 3.92 (s, 6H, 2CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 158.9 (2C-3,5), 155.0 (C-1), 91.8 (C-4), 65.9 (2C-2,6) 56.4 (2CH$_3$). MS (ESI), $m/z$ (%): 406.1 (100) [M + H]$^+$.

*4.2. Ethyl (2,6-diiodo-3,5-dimethoxyphenoxy) acetate (3).* To a solution of 2,6-diiodo-3,5-dimethoxyphenol (**2**) (1.00 g, 2.46 mmol) in N,N-dimethylformamide (3 mL) was added sodium hydride 60% (148 mg, 3.70 mmol) at room temperature. When no more gas evolved, ethyl bromoacetate (0.4 mL, 3.70 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 12 h. After

addition of water, the aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography using cyclohexane/ethyl acetate (9/1) as an eluent system to give compound **3** as a white solid (871 mg, 72%). $R_f = 0.27$ (cyclohexane/ethyl acetate: 8/2). Mp = 112–113°C. IR (cm$^{-1}$): 1751, 1566, 1334, 1107, 738. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 6.32 (s, 1H, H-4), 4.61 (s, 2H, OCH$_2$), 4.35 (q, J = 7.2 Hz, 2H, CH$_2$CH$_3$), 3.91 (s, 6H, 2CH$_3$), 1.37 (t, J = 7.1 Hz, 3H, CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 167.7 (C=O), 159.3 (2C-3,5), 158.2 (C-1), 95.9 (C-4), 73.6 (2C-2,6), 68.7 (CH$_2$CO), 61.3 (CH$_2$CH$_3$), 56.2 (2CH$_3$), 14.2 (CH$_3$). MS (ESI), $m/z$ (%): 493.1 (100) [M + H]$^+$.

*4.3. Ethyl (2,6-dicyano-3,5-dimethoxyphenoxy) acetate (4).* A mixture of ethyl (2,6-diiodo-3,5-dimethoxyphenoxy) acetate (**3**) (800 mg, 1.63 mmol) and copper (I) cyanide (394 mg, 4.4 mmol) in N,N-dimethylformamide (3 mL) was heated at 160°C for 2 h. After cooling, the reaction mixture was poured into crushed ice, and a precipitate was formed, which was extracted with ethyl acetate (2 × 30 mL). The organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated to dryness. The resulting oil was purified by silica gel column chromatography using cyclohexane/ethyl acetate (8/2) as eluent to give compound **4** as a white solid (355 mg, 75%). $R_f = 0.60$ (cyclohexane/ethyl acetate: 6/4); Mp = 65–66°C. IR (cm$^{-1}$): 2924, 2222, 1755, 1597, 1498, 1381, 1176, 1122. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 6.26 (s, 1H, H-4), 5.13 (s, 2H, COCH$_2$), 4.28 (q, 2H, J = 7.1 Hz, CH$_2$CH$_3$), 3.91 (s, 6H, 2CH$_3$), 1.30 (t, 3H, J = 7.1 Hz, CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 167.3 (C=O), 165.4 (2C-3,5), 164.4 (C-1), 112.5 (2C≡N), 93.1 (C-4), 88.7 (2C-2,6), 69.1 (CH$_2$CO), 61.9 (CH$_2$CH$_3$), 56.4 (2CH$_3$), 14.0 (CH$_3$). MS (ESI), $m/z$ (%): 291.1 (100) [M + H]$^+$.

*4.4. Ethyl 3-amino-7-cyano-4,6-dimethoxy-1-benzofuran-2-carboxylate (5).* To a solution of ethyl (2,6-dicyano-3,5-dimethoxyphenoxy) acetate (**4**) (300 mg, 1.03 mmol) in N,N-dimethylformamide (3 mL) was added sodium hydride 60%

(84 mg, 2.1 mmol) at 0°C. The mixture was stirred for 30 min, quenched with water, and extracted with ethyl acetate (100 mL). The organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated to dryness. The resulting oil was purified by silica gel column chromatography using cyclohexane/ethyl acetate (8/2) as an eluent system to give compound **5** as a beige solid (153 mg, 51% yield). $R_f = 0.71$ (cyclohexane/ethyl acetate: 1/1); Mp = 124–125°C. IR (cm$^{-1}$): 2223, 1614, 1554, 1508, 1454, 1338, 1305, 1103, 966, 700. $^1$H NMR (400 MHz, DMSO-d$_6$), $\delta$ ppm: 6.98 (s, 1H, H-5), 5.90 (s, 2H, NH$_2$), 4.32 (q, 2H, $J = 7.1$ Hz, CH$_2$CH$_3$), 3.91 (s, 6H, 2CH$_3$), 1.34 (t, 3H, $J = 7.1$ Hz, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$), $\delta$ ppm: 163.9 (C=O), 160.8 (C-6), 158.6 (C-4), 155.4 (C-3), 124.6 (C-2), 112.6 (C≡N), 106.3 (C), 92.0 (C-5), 80.5 (C-7), 60.1 (CH$_2$CH$_3$), 56.7 (CH$_3$), 56.0 (CH$_3$), 53.4 (C), 14.6 (CH$_3$). MS (ESI), $m/z$ (%): 291.2 (100) [M + H]$^+$.

### 4.5. 4-Hydroxy-7,9-dimethoxy[1]benzofuro[3,2-d]pyrimidine-6-carbonitrile (6).

A mixture of ethyl 3-amino-7-cyano-4,6-dimethoxy-1-benzofuran-2-carboxylate (**5**) (100 mg, 0.34 mmol) in triethyl orthoformate (2 mL) was irradiated in a microwave at 200°C for 15 min. The resultant yellow solution was evaporated to dryness, and the residue was dissolved in a solution of ammonia in methanol 7N (2 mL). The mixture was irradiated in a microwave at 140°C for 15 min, and the resultant precipitate was collected and evaporated to dryness. The residue was triturated with acetonitrile, and the solid was collected by filtration. The solids were combined to give compound **6** as a white solid (43 mg, 46%). $R_f = 0.65$ (ethyl acetate); Mp = 268–269°C. IR (cm$^{-1}$): 3182, 2222, 1685, 1598, 1487, 1433, 1366, 1116, 1093, 1022, 962, 692. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 13.03 (s, 1H, OH), 8.27 (s, 1H, H-2), 7.20 (s, 1H, H-8), 4.05 (s, 3H, CH$_3$), 3.93 (s, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 164.1 (C-4), 159.2 (C-9), 155.9 (C-7), 154.6 (C), 153.3 (C), 147.6 (C-2), 145.3 (C), 112.6 (C≡N), 105.0 (C-6), 104.9 (C), 91.9 (C-8), 56.6 (CH$_3$), 56.1 (CH$_3$). MS (ESI), $m/z$ (%): 272.1 (100) [M + H]$^+$.

### 4.6. 2-Hydroxy-4,6-dimethoxybenzamide (8).

To a stirred suspension of 3,5-dimethoxyphenol (**1**) (4.00 g, 26.00 mmol) in acetonitrile (40 mL) at 0°C under argon was added chlorosulfonyl isocyanate (3.4 mL, 39.00 mmol). The mixture was maintained at this temperature for 10 min; then, the reaction mixture was quenched with hydrochloric acid 5 M (40 mL). After 10 h at room temperature, the reaction was diluted with water (100 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography using cyclohexane/ethyl acetate (8/2) as an eluent system to give compound **8** as a white solid (2.30 g, 45%). $R_f = 0.46$ (cyclohexane/ethyl acetate: 6/4); Mp = 154–155°C. IR (cm$^{-1}$): 3429, 3209, 1620, 1587, 1492, 1415, 1355, 1315, 1294, 1217, 1199, 1159, 1109, 1041. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 14.69 (s, 1H, OH), 8.00 (d, $J = 3.20$ Hz, 2H, CONH$_2$), 6.08 (d, $J = 2.50$ Hz, 1H, H-5), 6.05 (d, $J = 2.40$ Hz, 1H, H-3), 3.87 (s, 3H, CH$_3$), 3.77 (s, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 171.7 (C = O), 165.9 (C-2), 163.7 (C-6), 160.2 (C-4), 96.6 (C-1), 94.1 (C-3), 90.1 (C-5), 56.0 (CH$_3$), 55.3 (CH$_3$). MS (ESI), $m/z$ (%): 198.1 (100) [M + H]$^+$.

### 4.7. 2-Hydroxy-3-iodo-4,6-dimethoxybenzamide (9).

A solution of 2-hydroxy-4,6-dimethoxybenzamide (**8**) (2.00 g, 10.15 mmol) in dichloromethane (40 mL) at 0°C under argon was added N-iodosuccinimide (2.28 g, 10.15 mmol), and the reaction mixture was maintained at this temperature for 10 min. The reaction was then diluted with water (60 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography using cyclohexane/ethyl acetate (6/4) as an eluent system to give compound **9** as a white solid (1.97 g, 60%). $R_f = 0.21$ (cyclohexane/ethyl acetate: 6/4). Mp = 270–271°C. IR (cm$^{-1}$): 3439, 1602, 1579, 1415, 1396, 1336, 1286, 1215, 1120, 1078. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 15.74 (s, 1H, OH), 8.13 (d, $J = 1.2$ Hz, 2H, CONH$_2$), 6.30 (s, 1H, H-5), 3.97 (s, 3H, CH$_3$), 3.92 (s, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO) $\delta$: 171.3 (C=O), 163.9 (C-2), 162.1 (C-6), 161.2 (C-4), 97.1 (C-1), 87.5 (C-5), 67.1 (C-3), 56.5 (CH$_3$), 56.4 (CH$_3$). MS (ESI), $m/z$ (%): 324.0 (100) [M + H]$^+$.

### 4.8. 3-Cyano-2-hydroxy-4,6-dimethoxybenzamide (10).

A mixture of 2-hydroxy-3-iodo-4,6-dimethoxybenzamide (**9**) (1.00 g, 3.10 mmol) and copper (I) cyanide (465 mg, 5.20 mmol) in N,N-dimethylformamide (4 mL) was heated at 160°C for 6 h. After cooling, the reaction mixture was poured into crushed ice, and a precipitate was formed, which was extracted with ethyl acetate (100 mL). The organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated to dryness. The resulting oil was purified by silica gel column chromatography using dichloromethane/ methanol (9/1) as an eluent system to give compound **10** as a beige solid (262 mg, 38%). $R_f = 0.82$ (dichloromethane/ methanol: 9/1). Mp = 300–301°C. IR (cm$^{-1}$): 3477, 3367, 2220, 1651, 1583, 1483, 1450, 1427, 1344, 1209, 1126, 1070. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 16.21 (s, 1H, OH), 8.34 (d, $J = 6.4$ Hz, 2H, CONH$_2$), 6.32 (s, 1H, H-5), 4.03 (s, 3H, CH$_3$), 3.98 (s, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 171.0 (C=O), 168.4 (C-4), 165.4 (C-6), 164.3 (C-2), 113.8 (C≡N), 96.5 (C-1), 87.4 (C-5), 82.8 (C-3), 57.0 (CH$_3$), 56.7 (CH$_3$). MS (ESI), $m/z$ (%): 223.1 (100) [M + H]$^+$.

### 4.9. Ethyl (2-carbamoyl-6-cyano-3,5-dimethoxyphenoxy) acetate (11).

To a solution of 3-cyano-2-hydroxy-4,6-dimethoxybenzamide (**10**) (222 mg, 1.00 mmol) in N,N-dimethylformamide (2 mL) was added sodium hydride 60% (48 mg, 1.20 mmol) at room temperature. When no more gas evolved, ethyl bromoacetate (150 µL, 1.40 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. After addition of water, the aqueous layer was extracted with dichloromethane (100 mL). The combined

organic extracts were dried over sodium sulfate and filtered. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography using cyclohexane/ethyl acetate (8/2) as an eluent system to give compound **11** as a white solid (277 mg, 90%). $R_f = 0.72$ (dichloromethane/methanol: 9/1). $M_p = 85–86°C$. IR (cm$^{-1}$): 3377, 3186, 2218, 1645, 1600, 1562, 1469, 1390, 1354, 1332, 1222, 1107, 1020. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 7.83 (s, 1H, CONH$_2$), 7.65 (s, 1H, CONH$_2$), 6.89 (s, 1H, H-4), 4.82 (s, 2H, CH$_2$CO), 4.15 (q, $J = 7.10$ Hz, 2H, CH$_2$CH$_3$), 3.99 (s, 3H, CH$_3$), 3.92 (s, 3H, CH$_3$), 1.21 (t, $J = 7.10$ Hz, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 167.1 (C=O), 164.2 (C=O), 161.8 (C-3), 160.3 (C-5), 157.6 (C-1), 114.8 (C≡N), 113.6 (C-2), 94.6 (C-4), 87.9 (C-6), 69.8 (CH$_2$CO), 60.7 (CH$_2$CH$_3$), 57.0 (CH$_3$), 56.6 (CH$_3$), 13.9 (CH$_3$). MS (ESI), m/z (%): 309.2 (100) [M + H]$^+$.

*4.10. Ethyl 3-amino-7-carbamoyl-4,6-dimethoxy-1-benzofuran-2-carboxylate (12).* To a solution of ethyl (2-carbamoyl-6-cyano-3,5-dimethoxyphenoxy) acetate (**11**) (200 mg, 0.65 mmol) in *N,N*-dimethylformamide (2 mL) was added sodium hydride 60% in mineral oil (25 mg, 0.65 mmol) at 0°C. The mixture was stirred for 10 min, quenched with water, and extracted with ethyl acetate (100 mL). The organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated to dryness. The resulting oil was purified by silica gel column chromatography using cyclohexane/ethyl acetate (6/4) as an eluent system to give compound **12** as a white solid (134 mg, 67%). $R_f = 0.68$ (dichloromethane/methanol: 9/1). $M_p = 215–216°C$. IR (cm$^{-1}$): 3462, 3365, 2922, 1620, 1556, 1454, 1296, 1159, 1105, 1026. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 7.57 (d, $J = 5.2$ Hz, 2H, CONH$_2$), 6.54 (s, 1H, H-5), 6.22 (s, 2H, NH$_2$), 4.26 (q, $^3J = 7.10$ Hz, 2H, CH$_2$CH$_3$), 3.97 (s, 3H, CH$_3$), 3.91 (s, 3H, CH$_3$), 1.29 (t, $^3J = 7.10$ Hz, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 164.7 (C=O), 163.8 (C=O), 159.4 (C-4), 156.0 (C-6), 153.3 (C-2), 142.2 (C-3), 105.5 (C-7), 104.0 (C), 103.9 (C), 90.7 (C-5), 59.1 (CH$_2$CH$_3$), 56.7 (CH$_3$), 56.0 (CH$_3$), 14.5 (CH$_3$). MS (ESI), m/z (%): 309.2 (100) [M + H]$^+$.

*4.11. 4-Hydroxy-7,9-dimethoxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (7).* A mixture of ethyl 3-amino-7-carbamoyl-4,6-dimethoxy-1-benzofuran-2-carboxylate (**12**) (100 mg, 0.32 mmol) in triethyl orthoformate (2 mL) was irradiated using microwave heating at 200°C for 15 min. The resultant yellow solution was evaporated to dryness, and the residue was dissolved in a solution of ammonia in methanol 7N (2 mL). The mixture was then irradiated using microwave heating at 140°C for 15 min, and the resultant precipitate was collected and evaporated to dryness. The residue was triturated with methanol, and the solid was collected by filtration. The solids were combined to give compound **7** as a brown solid (58 mg, 62%). $R_f = 0.21$ (dichloromethane/methanol: 9/1). Mp = 262–263°C (methanol). IR (cm$^{-1}$): 3429, 3169, 1649, 1608, 1317, 1215, 1099, 1002. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 8.31 (s, 1H, H-2), 7.76 (s, 1H, CONH$_2$), 7.52 (s, 1H, CONH$_2$), 7.28 (s, 1H, OH), 6.71 (s, 1H, H-8), 4.05 (s, 3H, CH$_3$), 3.95 (s, 3H, CH$_3$). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 164.0 (C=O), 159.2 (C-9), 156.0 (C-7), 154.7 (C), 153.4 (C-2), 148.9 (C), 145.3 (C-4), 133.1 (C), 105.1 (C), 105.0 (C-6), 91.9 (C-8), 56.7 (CH$_3$), 56.2 (CH$_3$). MS (ESI), m/z (%): 290.1 (100) [M + H]$^+$. Anal. calcd for C$_{13}$H$_{11}$N$_3$O$_5$: C 53.98, H 3.83, N 14.53. Found: C 53.92, H 3.79, N 14.50.

*4.12. 4,7,9-Trihydroxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (13).* A mixture of 4-hydroxy-7,9-dimethoxy [1]benzofuro[3,2-d]pyrimidine-6-carboxamide (**7**) (50 mg, 0.17 mmol) in warm pyridine hydrochloride (3 g) was irradiated using microwave (100 W) heating at 200°C for 10 min. After addition of water, the mixture was extracted with ethyl acetate (100 mL). The organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated to dryness. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (9/1) as an eluent system to give compound **13** as a yellow solid (19 mg, 43%). $R_f = 0.41$ (dichloromethane/methanol: 9/1). Mp = 298–299°C (methanol). IR (cm$^{-1}$): 3464, 3072, 2883, 1604, 1402, 1269, 1195, 1120. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 14.03 (s, 1H, OH), 12.89 (s, 1H, OH), 11.50 (s, 1H, OH), 8.40 (s, 1H, CONH$_2$), 8.21 (s, 1H, H-2), 7.44 (s, 1H, CONH$_2$), 6.36 (s, 1H, H-8). $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 169.6 (C=O), 166.2 (C-4), 158.2 (C-7), 156.1 (C-9), 151.4 (C), 146.9 (C-2), 144.0 (C), 136.8 (C), 104.0 (C), 99.1 (C-8), 92.9 (C). MS (ESI), m/z (%): 262.0 (100) [M + H]$^+$. Anal. calcd for C$_{11}$H$_7$N$_3$O$_5$: C 50.58, H 2.70, N 16.09. Found: C 50.42, H 2.71, N 16.13.

## Data Availability

The data used to support the findings of this study are included within the article and in Supplementary Materials.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

## Supplementary Materials

S1: $^1$H NMR of 2,6-diiodo-3,5-dimethoxyphenol (**2**). S2: $^{13}$C NMR of 2,6-diiodo-3,5-dimethoxyphenol (**2**). S3: $^1$H NMR of ethyl (2,6-diiodo-3,5-dimethoxyphenoxy) acetate (**3**). S4: $^{13}$C NMR of ethyl (2,6-diiodo-3,5-dimethoxyphenoxy) acetate (**3**). S5: $^1$H NMR of ethyl (2,6-dicyano-3,5-dimethoxyphenoxy) acetate (**4**). S6: $^{13}$C NMR of ethyl (2,6-dicyano-3,5-dimethoxyphenoxy) acetate (**4**). S7: $^1$H NMR of ethyl

3-amino-7-cyano-4,6-dimethoxy-1-benzofuran-2-carboxylate (**5**). S8: $^{13}$NMR of ethyl 3-amino-7-cyano-4,6-dimethoxy-1-benzofuran-2-carboxylate (**5**). S9: $^{1}$H NMR of 4-hydroxy-7,9-dimethoxy[1]benzofuro[3,2-d]pyrimidine-6-carbonitrile (**6**). S10: $^{13}$C NMR of 4-hydroxy-7,9-dimethoxy [1]benzofuro[3,2-d]pyrimidine-6-carbonitrile (**6**). S11: $^{1}$H NMR of 2-hydroxy-4,6-dimethoxybenzamide (**8**). S12: $^{13}$C NMR of 2-hydroxy-4,6-dimethoxybenzamide (**8**). S13: $^{1}$H NMR of 2-hydroxy-3-iodo-4,6-dimethoxybenzamide (**9**). S14: $^{13}$C NMR of 2-hydroxy-3-iodo-4,6-dimethoxybenzamide (**9**). S15: $^{1}$H NMR of 3-cyano-2-hydroxy-4,6-dimethoxybenzamide (**10**). S16: $^{13}$C NMR of 3-cyano-2-hydroxy-4,6-dimethoxybenzamide (**10**). S17: $^{1}$H NMR of ethyl (2-carbamoyl-6-cyano-3,5-dimethoxyphenoxy) acetate (**11**). S18: $^{13}$C NMR of ethyl (2-carbamoyl-6-cyano-3,5-dimethoxyphenoxy) acetate (**11**). S19: $^{1}$H NMR of ethyl 3-amino-7-carbamoyl-4,6-dimethoxy-1-benzofuran-2-carboxylate (**12**). S20: $^{13}$C NMR of ethyl 3-amino-7-carbamoyl-4,6-dimethoxy-1-benzofuran-2-carboxylate (**12**). S21: $^{1}$H NMR of 4-hydroxy-7,9-dimethoxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (**7**). S22: $^{13}$C NMR of 4-hydroxy-7,9-dimethoxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (**7**). S23: $^{1}$H NMR of 4,7,9-trihydroxy[1]benzofuro[3,2-d] pyrimidine-6-carboxamide (**13**). S24: $^{13}$C NMR of 4,7,9-trihydroxy[1]benzofuro[3,2-d]pyrimidine-6-carboxamide (**13**). . (*Supplementary Materials*)

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