# SYNTHESIS, STRUCTURAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NEW PYRIDINE BENZAMIDES

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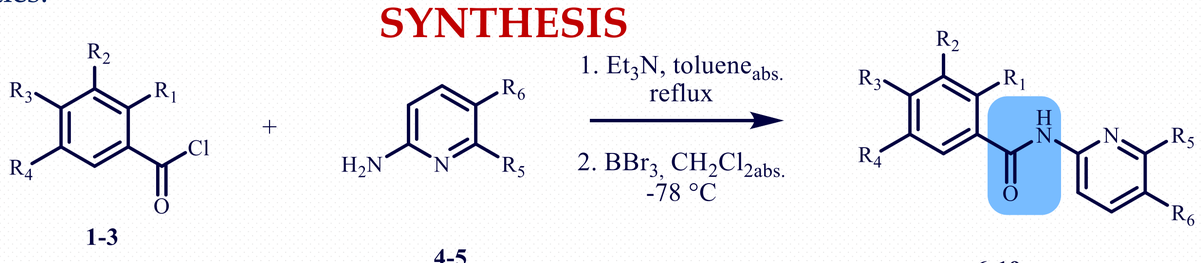
## INTRODUCTION

Amide bonds are ubiquitous structural motifs present in natural products, peptides, agrochemicals, functional materials, and pharmaceutical agents. Their broad occurrence and unique physicochemical properties make amide-containing molecules indispensable precursors and intermediates in the synthesis of pharmaceuticals and other useful chemical entities. Pyridine derivatives exhibit a wide range of biological activities, making them valuable scaffolds in medicinal chemistry and drug development. Among these, pyridylbenzamides constitute an important class of nitrogen-containing heterocyclic amides that have attracted considerable attention due to their diverse pharmacological properties.

## SYNTHESIS




**1-3**

**1** $R_1 = R_2 = R_3 = R_4 = H$
**2** $R_1 = R_3 = OCH_3$, $R_2 = R_4 = H$
**3** $R_1 = H$, $R_2 = R_3 = R_4 = OCH_3$

**4-5**

**4** $R_5 = H$, $R_6 = OCH_3$
**5** $R_5 = R_6 = OCH_3$

**6-19**

**6** $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $R_6 = OCH_3$
**7** $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = R_6 = OCH_3$
**8** $R_1 = R_3 = R_5 = R_6 = OCH_3$, $R_2 = R_4 = H$
**9** $R_1 = R_5 = H$, $R_2 = R_3 = R_4 = R_6 = OCH_3$
**10** $R_1 = H$, $R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$
**11** $R_1 = OCH_3$, $R_2 = R_4 = H$, $R_3 = R_5 = R_6 = OH$
**12** $R_1 = R_3 = R_6 = OH$, $R_2 = R_4 = H$, $R_5 = OCH_3$
**13** $R_1 = R_5 = R_6 = OH$, $R_2 = R_4 = H$, $R_3 = OCH_3$
**14** $R_1 = R_3 = OCH_3$, $R_2 = R_4 = H$, $R_5 = R_6 = OH$
**15** $R_1 = R_3 = R_5 = R_6 = OH$, $R_2 = R_4 = H$
**16** $R_1 = R_5 = H$, $R_2 = R_3 = R_4 = R_6 = OH$
**17** $R_1 = H$, $R_2 = R_3 = R_4 = R_5 = R_6 = OH$
**18** $R_1 = H$, $R_2 = R_3 = R_4 = R_6 = OH$, $R_5 = OCH_3$
**19** $R_1 = H$, $R_2 = R_3 = R_4 = OH$, $R_5 = R_6 = OCH_3$

Benzamides were prepared from corrersponding benzoyl chlorides and 2-aminopyridines. Targeted hydroxy substituted benzamides obtained by demethylation of methoxy derivatives with $BBr_3$. The structures of the prepared compounds were characterized by menas of 1D and 2D NMR spectroscopy and MS spectrometry.

**Scheme 1.** Synthesis of benzamides **6–19**

## NMR SPECTROSCOPIC CHARACTERIZATION

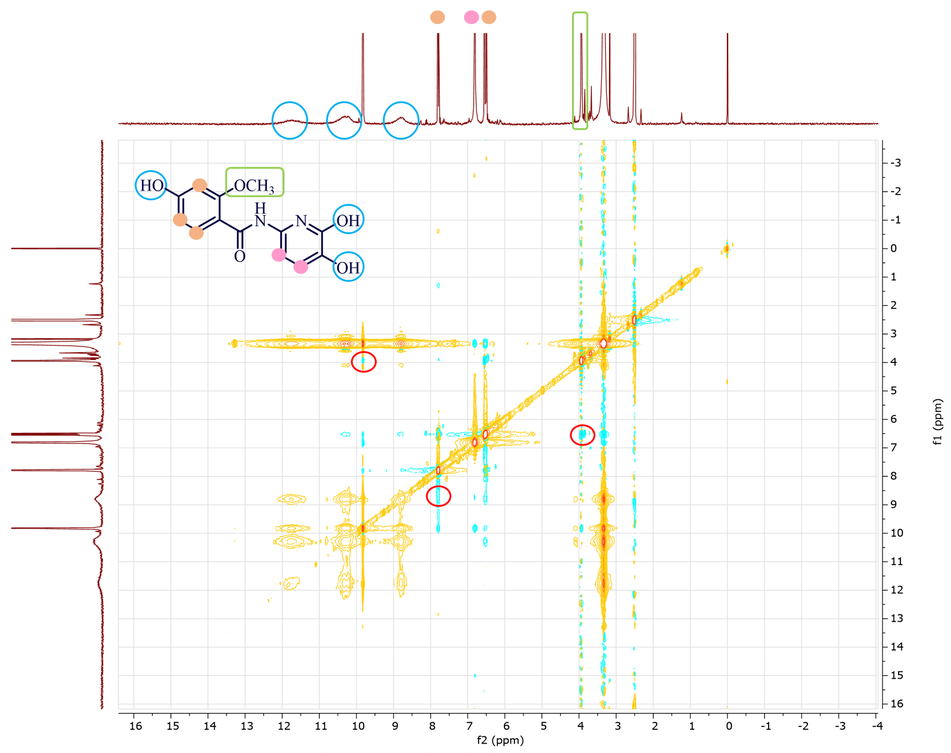



**Figure 1.** 2D NMR spectrum of **11**

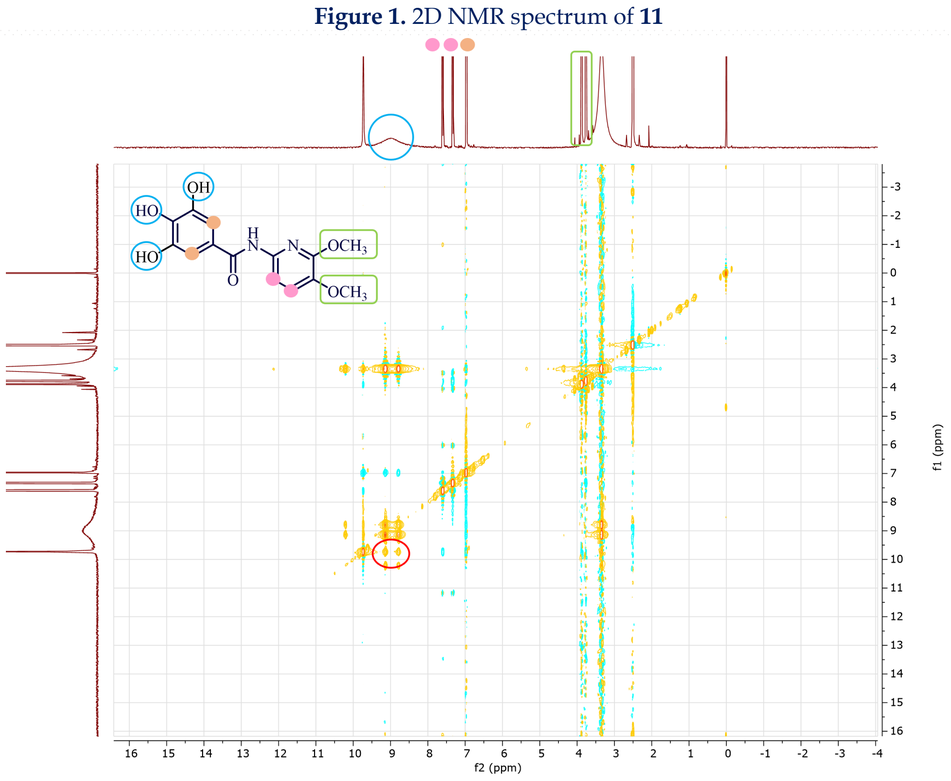



**Figure 2.** 2D NMR spectrum of **19**

Based on the NOE interactions between the amide and methoxy groups, as well as the interaction of the proton at position 3 of the phenyl ring with only one hydroxy group, we conclude that demethylation occurs at position 4 of the phenyl ring and at positions 5 and 6 of the pyridine ring (Figure 1). The presence of two signals for methoxy groups indicates that the methoxy groups are not located at positions 3 and 5 of the phenyl ring. Furthermore, the pyridine doublets show no NOE interaction with the OH group as well as signals of methoxy groups, suggesting that demethylation occurs exclusively on the phenyl ring (Figure 2).

## ANTIPROLIFERATIVE ACTIVITY *IN VITRO*

The antiproliferative activity of the synthesized benzamides **6-19** was evaluated in a several human cancer cell lines according to the Table 1 (LN-229, Capan-1, HCT-116, NCI-H460, HL-60, K-562, Z-138, MOLT-4 and SH-SY5Y). Etoposide (ETO) as a standard antitumor drug was evaluated for comparison of obtained results. Table 1 presents results expressed as $IC_{50}$ values.

**Table 1.** Antiproliferative activity of benzamides **6–19**

| Cpd. | $IC_{50}$ (μM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Capan-1 | HCT-116 | LN229 | NCI-H460 | Molt-4 | HL-60 | K562 | SH-SY5Y | Z138 | PBMC |
| **6** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **7** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **8** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **9** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **10** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **11** | >100 | 94.32 ± 3.60 | 84.69 ± 1.61 | >100 | >100 | >100 | >100 | >100 | 85.87 ± 1.62 | >100 |
| **12** | >100 | 71.22 ± 21.18 | 78.77 ± 0.79 | >100 | >100 | >100 | >100 | >100 | 76.42 ± 0.52 | >100 |
| **13** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **14** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **15** | 97.24 ± 2.76 | **24.15 ± 5.28** | **17.85 ± 0.28** | 87.26 ± 0.79 | >100 | 96.57 ± 3.62 | >100 | >100 | **18.08 ± 0.18** | >100 |
| **16** | >100 | 63.33 ± 27.78 | 84.42 ± 0.32 | 84.96 ± 1.32 | >100 | >100 | >100 | >100 | **33.44 ± 1.41** | >100 |
| **17** | 94.14 ± 0.20 | **22.91 ± 4.65** | **13.98 ± 4.94** | 84.82 ± 0.48 | >100 | >100 | >100 | >100 | **15.87 ± 3.28** | >100 |
| **18** | >100 | **38.69 ± 0.83** | **17.15 ± 0.30** | 83.31 ± 1.12 | >100 | 98.44 ± 1.56 | >100 | >100 | **20.75 ± 1.76** | >100 |
| **19** | >100 | **35.95 ± 0.47** | **16.67 ± 1.68** | 81.95 ± 0.15 | >100 | 81.22 ± 18.78 | >100 | >100 | **32.33 ± 2.81** | >100 |
| **ETO** | 0.35 | 1.70 | 1.20 | 0.60 | - | 0.25 | 0.60 | - | 0.35 | 5.70 |

## ANTIOXIDATIVE ACTIVITY *IN VITRO*

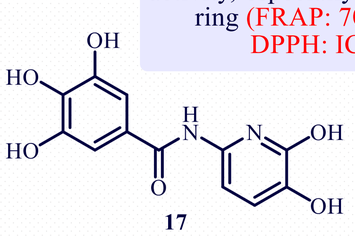
Newly prepared compounds were also tested for their antioxidative activity *in vitro* by using three spectroscopic methods – DPPH, ABTS and FRAP (Table 2). Most compounds showed very good to strong activity, particularly compounds **14–19** in the FRAP assay, **14** and **16–19** in the DPPH assay, and compounds **13** and **15** in the ABTS assay.

**Table 2.** Antioxidative activity of benzamides **6–19**

| Cpd. | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | BHT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FRAP (mmol $Fe^{2+}$/mmolC) | 4.950±0.10 | 7.230±0.61 | 117.330±1.91 | 5.090±0.20 | 66.130±1.76 | **2080.690±37.79** | - | **1977.360±108.34** | **2381.760±35.27** | **2305.150±62.99** | **3411.470±15.12** | **7049.290±20.16** | **5298.070±138.57** | **2714.900±7.56** | 2089.340 |
| DPPH ($IC_{50}$/M) | - | - | - | - | $4.240x10^{-3}$± $3.60x10^{-4}$ | $4.175x10^{-6}$± $3.43x10^{-7}$ | - | $7.351x10^{-6}$± $1.45x10^{-7}$ | **$1.284x10^{-5}$± $6.51x10^{-6}$** | $3.667x10^{-6}$± $2.83x10^{-7}$ | **$1.912x10^{-6}$± $3.75x10^{-8}$** | **$1.326x10^{-6}$± $2.04x10^{-7}$** | **$1.862x10^{-6}$± $2.50x10^{-7}$** | **$1.862x10^{-6}$± $2.50x10^{-7}$** | $2.500x10^{-5}$ |
| ABTS ($IC_{50}$/M) | - | **$2.200x10^{-4}$± $6.65x10^{-6}$** | - | - | - | **$2.238x10^{-4}$± $1.44x10^{-5}$** | - | **$1.082x10^{-4}$± $1.06x10^{-6}$** | $3.393x10^{-5}$± $6.22x10^{-7}$ | **$1.927x10^{-4}$± $6.58x10^{-6}$** | $3.255x10^{-5}$± $1.27x10^{-6}$ | $3.753x10^{-5}$± $4.45x10^{-7}$ | $4.723x10^{-5}$± $1.20x10^{-7}$ | $3.682x10^{-4}$± $6.07x10^{-5}$ | $2.300x10^{-5}$ |

## CONCLUSIONS

- The most of tested compounds showed moderate antiproliferative activity on the tested human cancer cell lines, while some of them showed **good activity and selectivity against LN229 and Z138**
- The obtained results showed that **the presence of hydroxy groups significantly improves antioxidative activity**, especially when they are located on the pyridine ring (Figure 3)
- Compounds **17** and **18 showed the most potent antioxidative activity** in the FRAP assay, compound **14** in the DPPH assay, and compound **13** in the ABTS assay




**17**

The presence of hydroxy groups improves antioxidative activity, especially when they are located on the pyridine ring (FRAP: 7049.29±20.16 mmol $Fe^{2+}$/mmolC; DPPH: $IC_{50}$=$1.326x10^{-6}$±$2.04x10^{-7}$M)

moderate activity and selectivity against LN229 and Z138

**Figure 3.** Structure of the compound **17**

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