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Dedicated to the memory of
Dr. Emilian GEORGESCU (1946-2020)

DESIGN AND SYNTHESIS OF NEW HYBRID PYRIDINE-IMIDAZOLIUM/BENZIMIDAZOLIUM SALTS WITH ANTIBACTERIAL ACTIVITY

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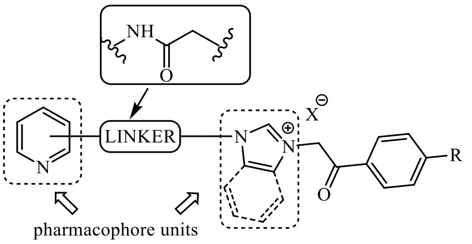
We report here the design and synthesis of newly hybrid salts with pyridine and imidazole / benzimidazole scaffolds. The pharmacophore units of hybrid compounds are the pyridine nucleus and imidazole / benzimidazole moieties, which are connected *via* an aliphatic linker consisting in an amide group and a methylene fragment. The synthesis of hybrid salts were performed in three steps: *N*-acylation, *N*-alkylation and quaternization. The synthesized hybrid compounds were characterized by spectral analysis (FT-IR, NMR). The preliminary antibacterial assay reveals that the hybrid salts have a very good antibacterial activity against both gram positive and gram negative germs.



INTRODUCTION

The azaheterocyclic derivatives, especially pyridine and imidazole/benzimidazole compounds, are reported as highly valuable scaffolds in modern medicinal chemistry having a large variety of biological actions, such as antibacterial, antifungal, antituberculosis, anticancer, anti-inflammatory, antihypertensive, anticoagulants, diuretics, antidepressant, etc.[1-7]

On the other hand, pyridine and imidazole/benzimidazole quaternary salts are valuable intermediates in the cycloimmonium chemistry for a large variety of synthesis, these including acylation, alkylation, quaternization, Huisgen 3+n dipolar cycloadditions reactions, etc.[1,8-16] Having in view the above considerations, obtaining of new azaheterocycles for biomedical applications continue to arouse great interest from pharmaceutical industry, one of the most important directions in the design of biologically active compounds being the combination of at least two pharmacophore units (one electron-rich and another π-deficient) in order to develop new hybrid bioactive molecules for medicinal chemistry usage.[1, 17-19]

RESULTS AND DISCUSSION

The pharmacophore units of the presented hybrid compounds are pyridine (a six membered ring azaheterocycle, π-deficient) combined with imidazole and benzimidazole (electron-rich azaheterocycles); the spacing element is an aliphatic

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chain and consists of an amide group and a methylene fragment. In the hybrid salts, an acetophenone moiety is anchored on the imidazole moiety, having various substituents grafted onto the *para* position of the benzene nucleus, the *p*-R-acetophenone moiety being a well known pharmacophore for antimicrobial and anticancer biological activity.[20-24] (Figure 1)

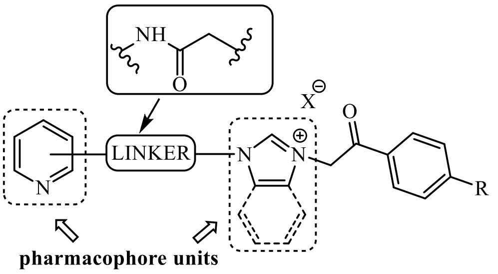
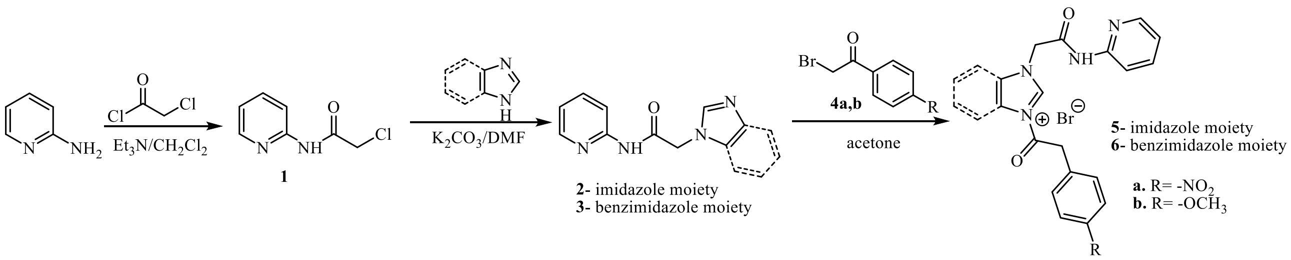


Fig. 1 – The design of hybrid salts.

The designed hybrid quaternary salts were obtained using three synthetic steps: *N*-acylation, *N*-alkylation and quaternization of the *N*^{3} nitrogen atom from the imidazole/benzimidazole ring. Thus, *N*-acylation of 2-aminopyridine with chloroacetyl chloride leads to the corresponding pyridine-acylamine **1**, which was previously reported.[1] Subsequent *N*-alkylation of the nitrogen atom (–NH– unit) of imidazole or benzimidazole, respectively, with pyridine-acylamine **1** obtained previously, results in a first class of hybrid derivatives pyridine- imidazole/benzimidazole **2** and **3**. In the last step, the quaternization reaction of the *N*^{3} atom from imidazole/benzimidazole ring with *para* substituted phenacyl bromides (with nitro or methoxy groups) **4a,b**, leads to the formation of hybrid salts of imidazolium **5a,b** and benzimidazolium **6a,b**, respectively. (Scheme 1).

In order to assign the structure of the hybrid compounds **5a,b**, respectively **6a,b**, the NMR and FT-IR spectra were recorded. The structure of the hybrid salts was demonstrated using ^{1}H-NMR, ^{13}C-NMR and homonuclear (^{1}H-^{1}H)-COSY and heteronuclear (^{1}H-^{13}C)-HMQC and (^{1}H-^{13}C)-HMBC correlations, analyzes that confirm the proposed structures. The main data furnished by FT-IR and NMR spectral analysis are listed in Table 1.

In the FT-IR spectra of hybrid salts **5a,b** and **6a,b**, the most important absorption bands are those provided by the amide and carbonyl groups. Thus, the absorption band characteristic for the NH group occurs between 3324 cm^{-1} (for the hybrid salt with imidazole moiety) and 3392 cm^{-1} (for the hybrid salt with benzimidazole moiety). The absorption band of carbonyl ketone group appears around 1709 cm^{-1} (for the salts **5a** and **6a** with *p*-NO_2, in accordance with the electron-withdrawing effect of nitro group) and 1673 cm^{-1} (for the salts **5b** and **6b** with *p*-OCH_3, due to the electron-donating effect of methoxy group). For the carbonyl amide group the absorption bands are around 1602 cm^{-1} (salt **6b**) and 1678 cm^{-1} (salt **5b**).



Scheme 1 – The pathway for synthesis of new hybrid pyridine imidazolium/benzimidazolium salts **5a,b** and **6a,b**.

Table 1

Main spectral data of hybrid salts **5a,b** and **6a,b**

Salt	IR, cm^{-1}	H_7 (NH)	H_{11}	$H_{2'}$ (CH_2)	H_9 (CH_2)	$C_{1'}$ (C=O)	C_8 (C=O)
5a	3353 (ν_{NH}); 1712 (ν_{COketo}); 1668 ($\nu_{COamide}$)	11.12	9.14	6.20	5.40	190.6	164.6
5b	3324 (ν_{NH}); 1683 (ν_{COketo}); 1678 ($\nu_{COamide}$)	11.12	9.15	6.09	5.40	189.5	164.7
6a	3354 (ν_{NH}); 1707 (ν_{COketo}); 1667 ($\nu_{COamide}$)	11.24	9.72	6.52	5.72	190.5	164.3
6b	3392 (ν_{NH}); 1664 (ν_{COketo}); 1602 ($\nu_{COamide}$)	11.24	9.76	6.43	5.71	189.3	164.3

The most deshielded signal in the NMR spectra is that provided by the amide NH group which appears at 11.12 ppm (for imidazolium salts **5a,b**) and 11.24 ppm (for benzimidazolium salts **6a,b**). The following signal belongs to proton H_{11}, from the imidazole ring, which appears at 9.14 ppm (for salts with imidazole scaffold) and 9.72 ppm (for salts with benzimidazole scaffold).

This deshielding is due to the electron-withdrawing effect of nitrogen atoms N^{10} and N^{12}, between which is situated the H_{11} proton. The signals of the hydrogen atoms from the two methylene groups H_9 and $H_{2'}$ appear at unusually high chemical shifts for such protons, due to the inductive withdrawing effects of the carbonyl-ketone and carbonyl-amide groups and the nitrogen atoms from the imidazole unit, respectively. Thus the signal of protons $H_{2'}$ appears between 6.20 ppm and 6.52 ppm, and the signal of protons H_9 are around 5.56 ppm.

In the ^{13}C-NMR spectra, the most important signals are those of the carbonyl ketone and amide groups, respectively. The signal of carbonyl amide C_8 appears around 164.5 ppm, while the signal of carbonyl ketone $C_{1'}$ appears between 190.6 ppm (for the salts **5a** and **6a** with R= $-NO_2$) and 189.5 ppm (for the salts **5b** and **6b** with R= $-OCH_3$).

The obtained hybrid salts were preliminary tested for their antimicrobial activity using the Kirby-Bauer agar disk diffusion method.[25] The antibacterial assay reveals that the hybrid salt **6a** have a very good antibacterial activity against gram positive germ *Staphylococcus aureus* and gram negative germ *Escherichia coli* (having the diameter of the inhibition zone in the range of 30 mm) and higher that the control drug Nystatin. These results make us confident to continue in the future the antibacterial assay looking for good findings.

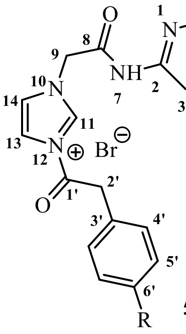
EXPERIMENTAL

General information

The reagents and solvents were purchased from commercial sources, being used without further purification. The melting points (uncorrected) were determined using an open capillary tubes introduced in a MEL-TEMP Electrothermal apparatus. FT-IR spectra were recorded in potassium bromide (KBr) pellets using a FTIR VERTEX 70 Bruker spectrometer. The NMR spectra have been determined on a Bruker AVANCE III 500 MHz spectrometer, operating at 500.19 and 125.7 MHz for ^{1}H and respectively ^{13}C nuclei. In ^{1}H and ^{13}C spectra, chemical shifts are reported in δ units (ppm) relative to the residual peak of solvent (ref: DMSO-d_6, ^{1}H: 2.50 ppm; ^{13}C: 39.52 ppm). The coupling constants (*J*) are given in Hz. In the NMR spectra to appoint the multiplicity of signals, the following abbreviations were used: s= singlet, d= doublet, t= triplet, m= multiplet, bs= broad singlet, ad= apparent doublet, add= apparent doublet of doublets, aq= apparent quartet.

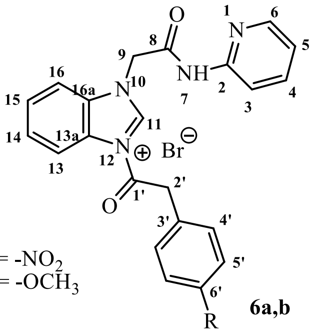
General procedure for synthesis of hybrid salts

The hybrid imidazolium/benzimidazolium salts were obtained by the following procedure: the intermediates **2** and **3** (1 mmol) were solubilized in about 20 mL of acetone (minimum amount) and then gradually add the different substituted phenacyl bromides (**4a,b**) (1.1 mmol), previously solubilized in 10-13 mL acetone. Reactions take place by stirring at room temperature (48-72 h), to give the corresponding hybrid quaternary salts **5a,b** and **6a,b**. The obtained salts were filtered off, washed two times with the same solvent (5 mL) and dried *in vacuum*. No other purification required.



5a,b

a. R= $-NO_2$
b. R= $-OCH_3$



6a,b

3-(2-(4-nitrophenyl)acetyl)-1-(2-oxo-2-(pyridin-2-ylamino) ethyl)-1H-imidazol-3-ium bromide (**5a**); Light yellow powder; η= 37%; m.p.= 217-218ºC; FT-IR (KBr, ν (cm^{-1})): 3356, 3042, 2974, 1712, 1668, 1586, 1474, 1304; ^{1}H-NMR (500 MHz, DMSO-*d6*): δ= 5.40 (2H: H_9, s), 6.20 (2H: $H_{2'}$, s), 7.17-7.15 (1H: H_5, *J*= 6.0 Hz, t,), 7.75 (1H: H_{14}, bs), 7.87-7.81 (1H: H_4, *J*= 7.5 Hz, t), 7.87 (1H: H_{13}, bs), 8.03-8.01 (1H: H_3, *J*= 6.0 Hz, ad), 8.31-8.29 (2H: $2xH_{4'}$, *J*= 8.5 Hz, d), 8.38-8.37 (1H: H_6, *J*= 4.0 Hz, ad), 8.46-8.45 (2H: $2xH_{5'}$, *J*= 8.5 Hz, d), 9.14 (1H: H_{11}, s), 11.12 (1H: H_7, s); ^{13}C-NMR (125 MHz, DMSO-*d6*): δ= 51.57 (C_9), 55.94 ($C_{2'}$), 113.46 (C_3), 119.99 (C_5), 123.46 (C_{13}), 123.86 (C_{14}), 124.15 ($2xC_{5'}$), 129.65 ($2xC_{4'}$), 138.41 ($C_{3'}$), 138.53 (C_4), 138.72 (C_{11}), 148.25 (C_6), 150.56 ($C_{6'}$), 151.35 (C_2), 164.69 (C_8), 190.69 ($C_{1'}$).

3-(2-(4-methoxyphenyl)acetyl)-1-(2-oxo-2-(pyridin-2-ylamino) ethyl)-1H-imidazol-3-ium bromide (**5b**); White powder; η= 54%; m.p.= 216-217ºC; FT-IR (KBr, ν (cm^{-1})): 3324, 3029, 2934, 1683, 1678, 1580, 1234, 1163; ^{1}H-NMR (500 MHz, DMSO-*d6*): δ= 3.88 (3H: *p*-OCH_3, s), 5.40 (2H: H_9, s), 6.09 (2H: $H_{2'}$, s), 7.16-7.15 (3H: $2xH_{5'}$, H_5, m), 7.75 (1H: H_{14}, bs), 7.85-7.80 (2H: H_{13}, H_4, m), 8.05-8.02 (3H: $2xH_{4'}$, H_3, m), 8.37-8.36 (1H: H_6, *J*= 5.5 Hz, add), 9.15 (1H: H_{11}, s), 11.12 (1H: H_7, s); ^{13}C-NMR (125 MHz, DMSO-*d6*): δ= 51.51 (C_9), 55.16 ($C_{2'}$), 55.78 (*p*-OCH_3), 113.47 (C_3), 114.35 ($2xC_{5'}$), 119.98 (C_5), 123.52 (C_{14}), 123.66 (C_{13}), 126.55 ($C_{3'}$), 130.60 ($2xC_{4'}$), 138.51 (C_4), 138.74 (C_{11}), 148.24 (C_6), 151.35 (C_2), 164. 10 ($C_{6'}$), 164.73 (C_8), 189.52 ($C_{1'}$).

3-(2-(4-nitrophenyl)acetyl)-1-(2-oxo-2-(pyridin-2-ylamino) ethyl)-1H-benzo[d]imidazol-3-ium bromide (**6a**); Light yellow powder; η= 90%; m.p.= 223-225ºC; FT-IR (KBr, ν (cm^{-1})): 3354, 3026, 2987, 1707, 1667, 1537, 1338; ^{1}H-NMR (500 MHz, DMSO-*d6*): δ= 5.72 (2H: H_9, s), 6.52 (2H: $H_{2'}$, s), 7.18-7.16 (1H: H_5, aq), 7.73-7.68 (2H: H_{14}, H_{15}, m), 7.83-7.79 (1H: H_4, m), 7.99 (1H: H_3, bs), 8.13-8.09 (2H: H_{13}, H_{16}, m), 8.40-8.36 (3H: H_6, 2x$H_{4'}$, m), 8.50-8.48 (2H: 2x$H_{5'}$, *J*= 8.5 Hz, d), 9.72 (1H: H_{11}, s), 11.24 (1H: H_7, s); ^{13}C-NMR (125 MHz, DMSO-*d6*): δ= 49.44 (C_9), 53.80 ($C_{2'}$), 113.51 (C_3), 113.88 (C_{16}), 114.07 (C_{13}), 120.09 (C_5), 124.09 (2x$C_{4'}$), 126.77 (C_{15}), 126.90 (C_{14}), 129.91 (2x$C_{5'}$), 131.50 (C_{16a}), 131.55 (C_{13a}), 138.51 ($C_{3'}$), 138.55 (C_4), 144.61 (C_{11}), 148.27 (C_2), 150.61 ($C_{6'}$), 151.32 (C_6), 164.37 (C_8), 190.52 ($C_{1'}$).

3-(2-(4-metoxyphenyl)acetyl)-1-(2-oxo-2-(pyridin-2-ylamino) ethyl)-1H-benzo[d]imidazol-3-ium bromide **(6b)**; White powder; η= 72%; m.p.= 185-188ºC; FT-IR (KBr, ν (cm^{-1})): 3392, 3005, 2978, 1664, 1602, 1545, 1236, 1167; ^{1}H-NMR (500 MHz, DMSO-*d6*): δ= 3.91 (3H: *p*-OCH_3, s), 5.71 (2H: H_9, s), 6.43 (2H: $H_{2'}$, s), 7.20-7.16 (3H: H_5, 2x$H_{5'}$, m), 7.72-7.67 (2H: H_{14}, H_{15}, m), 7.83-7.79 (1H: H_4, aq), 7.99 (1H: H_3, bs), 8.12-8.06 (4H: H_{13}, H_{16}, 2 x $H_{4'}$, m), 8.40-8.39 (1H: H_6, *J*= 4.5 Hz, ad), 9.76 (1H: H_{11}, s), 11.24 (1H: H_7, s); ^{13}C-NMR (125 MHz, DMSO-*d6*): δ= 49.39 (C_9), 52.94 ($C_{2'}$), 55.83 (*p*-OCH_3), 113.51 (C_3), 113.83 (C_{16}), 113.93 (C_{13}), 114.34 (2x$C_{5'}$), 120.07 (C_5), 126.61 ($C_{3'}$), 126.71 (C_{15}), 126.80 (C_{14}), 130.90 (2x$C_{4'}$), 131.49 (C_{16a}), 131.59 (C_{13a}), 138.53 (C_4), 144.26 (C_{11}), 148.26 (C_6), 151.31 (C_2), 164.23 ($C_{6'}$), 164.38 (C_8), 189.31 ($C_{1'}$).

CONCLUSIONS

The new hybrid salts having two pharmacophore units (pyridine nucleus and imidazole/benzimidazole moieties), connected *via* an aliphatic linker are designed and synthesized. Using only three steps the synthesis of hybrid salts were done: *N*-acylation, *N*-alkylation and quaternization. The structure of the new quaternary salts was proved by spectral analysis (FT-IR, NMR). The preliminary antibacterial assay reveals that the hybrid salt **6a** have a very good antibacterial activity against both gram positive germ *Staphylococcus aureus* and gram negative germ *Escherichia coli*.

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