SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY EVALUATION OF NEW AGENTS FROM BENZAMIDES CLASS

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Abstract

The use of most antimicrobial agents is limited, not only by the rapid developement of drug resistance, but also by the unsatisfactory status of present treatment of bacterial infections and drug side effects. This paper is a continuation of our research performed in order to develop new fluoro-trifluoromethyl-substituted benzamides with an improved antimicrobial profile, which targets the ideal antiseptic characteristics. These new fluoro-trifluoromethyl-substituted N- (2 - diethylaminoethyl) - N - (2,6 - dimethylphenyl) - benzamides (hydrochlorides) were characterized in terms of spectral and antimicrobial action, presenting a broad spectrum of action that was correlated with molecular substitution.

Rezumat

Utilizarea multor agenţi antimicrobieni este limitată, nu numai din cauza dezvoltării rapide a rezistenţei la medicamente, dar, de asemenea, de insuficienţa tratamentului actual al infecţiilor bacteriene şi de efectele secundare ale acestor medicamente. Această lucrare este o continuare a cercetărilor noastre de a dezvolta noi benzamide fluoro-trifluormetil-substituite cu un profil antimicrobian îmbunătăţit, care se apropie de caracteristicile antisepticului ideal. Aceste noi N-(2-dietilaminoetil)-N-(2,6-diclorfenil)-benzamide (clorhidraţi) fluoro-trifluormetil-substituite au fost caracterizate spectral şi din punct de vedere al acţiunii antimicrobiene, prezentând un spectru larg de acţiune, care a fost corelat cu modul de substituţie moleculară.

Keywords: benzamides, N-(2-diethylaminoethyl)-N-(2,6-dimethyl phenyl)-benzamides, antimicrobial

Introduction

The search for new antimicrobial agents is a result of the increasing number of multiresistant pathological microorganisms. Therefore the development of new and different antimicrobial drugs is an important objective and much of research program efforts are directed towards the design of new agents.

A common target for antibacterial therapy is the process of peptidoglycan biosynthesis, the essential component of the bacterial wall. The mechanism of action of many antibiotics is to block one or more steps in the synthesis of extracellular peptidoglycan and it appers that this effect is also characteristic for amides because the amide group mimics the conformational link between two successive amino acids, serving as a false substrate for peptidases that contribute to peptidoglycan synthesis, resulting in a labile structure that it will not be able to perform the normal function of the pathogen agent defence [5].

The known benzamides which are used for their antiseptic properties, have the disadvantage of the impossibility of oral administration because of their irritative efect on the gastric mucosa due to salycilic acid derivative structure. They also have the disadvantage of a low solubility in water, the administration being difficult, and the number of pharmaceutical forms is reduced [14].

Having experience in synthesis of N-(2-dialkylaminoethyl)-N-(2,6-dimethylphenyl)benzamides [1, 6, 9-12], we obtained new fluoro- and trifluouromethyl-substituted derivatives as antimicrobial agents; they have the nitrogen atom from the amide group substituted with a dialkylaminoethyl chain, the salts (hydrochlorides) being easily obtained. These compounds have the advantage of a good solubility in water and they can be used as aqueous solution.

We opted for this type of substitution because it is known that the -CF_3 group plays an important role in the medicinal chemistry area because it often enhances the efficacy by promoting electrostatic interaction with targets, improving cellular membrane permeability and increasing resistance towards oxidative metabolism of the drug [7].

On the other hand, new fluoro-substituted analogs are currently being designed having the aim of increasing metabolic stability. As it is known in the literature [4], the incorporation of fluorine enhances therapeutic efficacy and improves pharmacological properties in bioactive molecules. The presence of fluorine often leads to increased lipid solubility, enhancing rates of absorption and transport of drugs *in vivo*.

Materials and Methods

Chemistry

All starting materials and solvents were purchased from common commercial suppliers and used without purification unless otherwise noted. Melting points are uncorrected and were measured in open capillary tubes on an Electrothermal 9100 apparatus. The elemental analyses were performed on a Perkin Elmer CHNS/O Analyser Series II 2400 apparatus.

The IR spectra were recorded on a JASCO FT/IR-4200 spectrometer with ATR PRO450-S accesory and diamond crystal. The NMR spectra were recorded on a Gemini 300BB instrument at room temperature, operating at 300 MHz for ^{1}H and 75.075 MHz for ^{13}C. For signals unequivocal assignment two dimensional correlation experiments were performed. The chemical shifts were recorded as δ values in parts per million (ppm) units, downfield to tetrametylsilane used as internal standard. The coupling constants values are reported in hertz and the splitting patterns are abbreviated as following: s, singlet; d, doublet; t, triplet; m-multiplet; b, broad.

The title compounds were prepared within a three step synthesis which consists in the alkylation reaction of 2,6-dimethylaniline (1) with N-(2-chloroethyl)-N,N-diethylamine hydrochloride (2), followed by the reaction of intermediary compounds with different fluoro- trifluoromethyl-substituted aromatic acid chlorides (4). The resulted benzamides (5) were turned into hydrochlorides by treating them with an etheric HCl solution, and then were purified by recrystallization from boiling ethyl acetate (Figure 1).

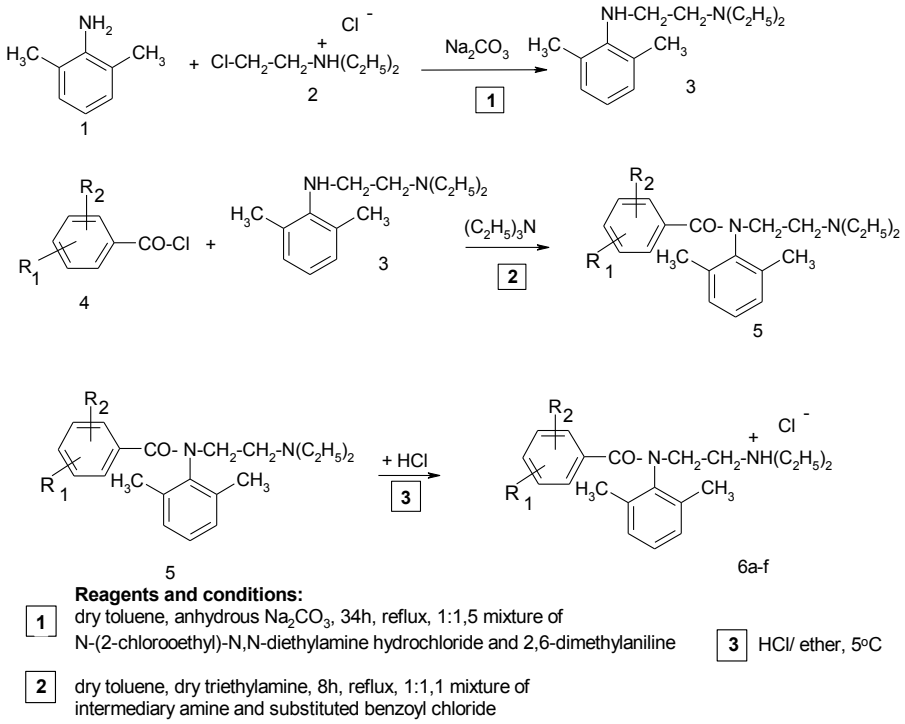


Figure 1
The synthesis scheme employed to obtain the target compounds

Synthesis of N-(2-diethylaminoethyl)-2,6-dimethylaniline (3)

In a round-bottom flask with three necks were placed 19.3 g (0.1125 mol) N-(2-chloroethyl)-N,N-diethylamine hydrochloride and 11.9 g (0.1125 mol) anhydrous Na_2CO_3 in 200 mL anhydrous toluene and then the mixture was heated under continous stirring at 105-110ºC for 3h; then 20.4 g (0.168 mol) 2,6-dimethylaniline dissolved in 50 mL anhydrous toluene were added and the refluxing was continued for 31 h. After the reaction mixture was cooled and filtered, 34 mL NaOH 5M were added and the organic layer was separated. The toluene solution was dried (anh. Na_2SO_4) and then evaporated in vacuum with a rotary evaporator, and the resulting amine was distilled under reduced pressure. We obtained 14.85 g N-(2-diethylaminoethyl)-2,6-dimethylaniline (0.0658 mol) as an yellow liquid with b.p. 161-164º C (8 mmHg) (η 58.43%).

General procedure for synthesis of benzamides (5)

In a round-bottom flask, 0.803 g (0.0036 mol) N-(2-diethylaminoethyl)-2,6-dimethylaniline were dissolved in 70 mL anhydrous toluene and mixed with 1.106 g (1.51 mL; 0.0109 mol) anhydrous triethylamine; 0.908 g (0.0040 mol) from the corresponding fluoro-trifluoromethyl-substituted acid chloride dissolved in 30 mL anhydrous toluene were added. The reaction mixture was refluxed for 8-10 h and after that was cooled and the precipitate of triethylamine hydrochloride was filtered, the toluene was removed by using a rotary evaporator and the residue was dissolved in 20 mL chloroform. The obtained solution was washed three times with 30 mL aqueous 10% Na_2CO_3 solution and once with water. The chloroformic solution was evaporated and the obtained benzamides were very viscous yellowish liquids which were purified by conversion in hydrochlorides.

General procedure for synthesis of N-(2-diethylaminoethyl)-N-(2,6-dimethylphenyl)benzamides (hydrochlorides) (6a-f).

The etheric solution of benzamide (5) in approximately 10 mL ether was cooled to 5-7ºC and then treated with an etherical HCl solution until the complete precipitation of the benzamide (6a-f). The resulting compounds (table 1) were purified by crystallization from ethyl acetate.

Antimicrobial evaluation

The synthesized compounds **6a-f** were evaluated for their *in vitro* antibacterial activity against the following bacterial strains: *Klebsiella pneumoniae*, *Escherichia coli ATCC-25922, Staphylococcus aureus ATCC-25923, Pseudomonas aeruginosa ATCC-27853, Enterococcus faecalis ATCC-28212*. The evaluation was performed according to the guidelines of the National Committee for Clinical Laboratory Standards [2,3,8,13]. The

qualitative screening of the susceptibility spectra of various microbial strains to the compounds **6a-f** was performed by adapted diffusion. The quantitative assay of the minimal inhibitory concentration (MIC, μg/mL) was based on liquid medium two-fold microdilutions. The MIC (μg/mL) was determined by binary micro dilution method, in 96 multi-well plates. The compounds were dissolved in water to give a concentration of 2048 μg/mL, which was serially diluted to give the following concentrations 1024, 512, 256, 128, 64, 32, 16 μg/mL in culture tubes containing 200 μL of nutrient medium. To all the tubes, 50 μL microbial inoculum was added and the tubes were incubated at 37 ± 1°C for 24 h. The MIC was recorded in each case as the minimum concentration of compound, which inhibited the growth of tested microorganism.

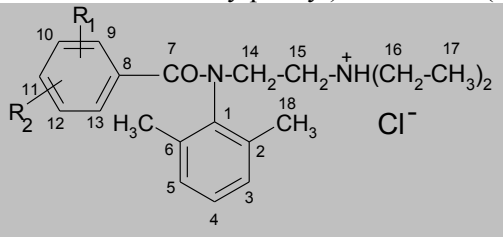
Results and Discussion

The obtained compounds are white or light-yellow crystals, with characteristic melting points (table I).

The compounds purity was certified by elemental analyses, the results being within ±0.4 of the theoretical values calculated for $C_{22}H_{27}ClF_4N_2O$(%): C, 59.12; H, 6.09; N, 6.27. Their chemical structures were elucidated by IR, ^{1}H-NMR, and ^{13}C-NMR analysis (Figure 2). In the table I are presented the reaction yields, melting points, elemental composition.

Table I

The new fluoro-trifluoromethyl-substituted N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-benzamide (hydrochloride) (6a-f)



$C_{22}H_{27}ClF_4N_2O$

446.91 g/ mol

Compd.	R_1	R_2	Elemental composition			m.p. (^{0}C)	η %
			C%	H%	N%		
6a	2-F	3-CF_3	59.32	6.14	6.35	153.5-155	47.64
6b	2-F	4-CF_3	59.04	6.12	6.30	135.1-137.2	53.81
6c	2-F	5-CF_3	59.23	6.03	6.22	133.2-136.5	44.22
6d	2-CF_3	5-F	59.22	6.20	6.35	153.6-156.1	38.65
6e	3-F	5-CF_3	58.99	6.14	6.14	172.2-176.4	46.63
6f	3-CF_3	4-F	59.02	6.11	6,17	144.3-145.1	42.12

N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-2-fluoro-3-trifluoromethyl-benzamide (hydrochloride) (6a)

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 12.36 (s, 1H, $N^{+}H$), 7.24 (bt, 7.7, 2H, H11-12), 7.03 (m, 1H, H-13), 6.98-7.10 (m, 3H, H3-5), 4.3 (m, 2H, H-14), 3.45 (m, 2H, H-15), 3.25 (m, 4H, H-16), 1.48 (t, 7.4, 6H, H-17), 2.25 (s, 6H, H-18)

^{13}C-NMR ($CDCl_3$, δ ppm, J Hz): 166.2 (C7), 156.4 (d, 260.2, C9), 138.5 (C1), 135.5 (C2,6), 132.2 (C12), 129.3 (C3,5), 129.0 (C4), 128.7 (C11), 125.2 (d, 14.8, C8), 123.7 (qd, 4.1, C13), 122.8 (q, 272.6, CF_3), 119.6 (dq, C10), 47.6 (C16), 47.4 (C15), 44.7 (C14), 8.6 (C17), 18.6 (C18)

FT-IR (solid in ATR, υ cm^{-1}): 2978.5, 2454.9, 1649.8, 1470.5, 1379.8, 1311.4, 1227.5, 1170.6, 1130.1, 1035.6, 808.0, 752.1, 688.5

N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-2-fluoro-4-trifluoromethyl-benzamide (hydrochloride) (6b)

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 12.42 (s, 1H, $N^{+}H$), 7.19-7.22m (3H, H10, H12-13), 6.98-7.10 (m, 3H, H3-5), 4.29 (m, 2H, H-14), 3.42 (m, 2H, H-15), 3.26 (m, 4H, H-16), 1.48 (t, 7.4, 6H, H-17), 2.26 (s, 6H, H-18)

^{13}C-NMR ($CDCl_3$, δ ppm, J Hz): 166.3 (C7), 158.7 (d, 253.4, C9), 138.4 (C1), 135.4 (C2,6), 134.4 (qd, 33.7, 8.0, C11), 129.4 (d, 19.7, C8), 129.3 (C3,5), 129.0 (C4), 127.9 (d, 15.5, C13), 123.3 (q, 275.4, CF_3), 120.4 (d, 3.7, C12), 47.5 (C16), 47.3 (C15), 44.6 (C14), 18.6 (C18), 8.6 (C17)

FT-IR (solid in ATR, υ cm^{-1}): 2976.6, 2565.8, 2468.4, 2349.8, 2318.0, 1647.9, 1510.0, 1470.5, 1419.4, 1376.0, 1322.0, 1258.3, 1211.1, 1174.4, 1121.4, 1063.6, 1035.6, 870.7, 834.1, 770.4, 744.4, 675.9

N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-2-fluoro-5-trifluoromethyl-benzamide (hydrochloride) (6c)

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 12.40 (s, 1H, $N^{+}H$), 7.04-7.33 (m, 3H, H-10,11,13), 6.98-7.10 (m, 3H, H3-5), 4.28(m, 2H, H-14), 3.41 (m, 2H, H-15), 3.25 (m, 4H, H-16), 1.46 (t, 7.4, 6H, H-17), 2.27 (s, 6H, H-18)

^{13}C-NMR ($CDCl_3$, δ ppm, J Hz): 166.4 (C7), 160.8 (d, 255.5, C9), 138.5 (C1), 135.5 (C2,6), 128.1 (C4), 127.9 (C11), 126.8 (C3,5), 123.2 (q, 270.6, CF_3), 122.2 (d, 19.4, C8), 121.4 (C-13), 116.9 (dq, 24.6, 3.7, C12), 115.6 (C10), 47.6 (C16), 47.4 (C15), 44.7 (C14), 18.7(C18), 8.7 (C17)

FT-IR (solid in ATR, υ cm^{-1}): 2975.6, 2431.8, 2121.3, 1646.0, 1532.2, 1473.4, 1387.5, 1312.3, 1268.0, 1163.8, 1116.6, 1071.3, 905.4, 832.1, 777.2, 686.5, 609.4

N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-5-fluoro-2-trifluoromethyl-benzamide (hydrochloride) (6d)

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 12.36 (s, 1H, $N^{+}H$), 7.85 (dd, 3.5; 8.9, 1H, H-13), 7.00-7.21 (m, 2H, H-10,11), 6.95-7.10 (m, 3H, H3-5), 4.30 (m,

2H, H-14), 3.39 (m, 2H, H-15), 3.25 (m, 4H, H-16), 1.47 (t, 7.4, 6H, H-17), 2.23 (s, 6H, H-18)

^{13}C-NMR ($CDCl_3$, δ ppm, J Hz): 166.2 (C7), 162.6 (254.8, C12), 139.1 (C1), 135.7 (d, 19.4, C8), 135.2 (C2,6), 134.8 (C11), 125.3 (C3,5), 124.6 (C4), 123.5 (q, 271.7, CF_3), 121.7 (C-13), 117.3 (C10), 117.0 (C9), 47.7 (C16), 47.2 (C15), 45.2 (C14), 18.6 (C18), 8.5 (C17)

FT-IR (solid in ATR, υ cm^{-1}): 2985.3, 2378.8, 1657.5, 1614.1, 1589.1, 1449.2, 1420.3, 1387.5, 1311.4, 1275.7, 1246.8, 1222.7, 1159.0, 1129.1, 1095.4, 1070.4, 1032.7, 893.8, 850.5, 815.7, 771.4, 654.7

N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-3-fluoro-5-trifluoromethyl-benzamide (hydrochloride) (6e)

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 12.42 (s, 1H, N^+H), 7.29 (bs, 1H, H-13), 7.24 (dt, 8.0, 2.0, 1H, H-9), 7.14 (m,1H, H-11), 6.94-7.10 (m, 3H, H3-5), 4.28 (m, 2H, H-14), 3.42 (m, 2H, H-15), 3.25 (m, 4H, H-16), 2.23 (s, 6H, H-18), 1.49 (t, 7.4, 6H, H-17)

^{13}C-NMR ($CDCl_3$, δ ppm, J Hz): 168.0 (C7), 161.5 (d, 250.5, C10), 139.6 (C1), 138.3 (d, 6.9, C8), 134.9 (C2,6), 132.9 (qd, 33.8; 7.7, C12), 129.8 (C3,5), 129.3 (C4), 123.2 (q, 272.8, CF_3), 121.7 (d, 3.7, C-13), 118.7 (d, 23.2, C9), 115.7dq (24.6, 3.7, C11), 47.6 (C15), 47.2 (C16), 45.3 (C14), 17.7 (C18), 8.6 (C17)

FT-IR (solid in ATR, υ cm^{-1}): 2977.6, 2427, 2361.4, 2341.2, 1641.1, 1598.7, 1540.9, 1466.6, 1418.4, 1387.5, 1347, 1306.5, 1276.7, 1217.8, 1172.5, 1133, 1091.5, 1018.2, 985.5, 956.5, 916, 878.4, 844.7, 822.5, 768.5, 737.6, 695.2, 669.2, 648.9, 617.1

N-(2-Diethylaminoethyl)-N-(2,6-dimethylphenyl)-4-fluoro-3-trifluoromethyl- benzamide (hydrochloride) (6f)

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 12.38 (s, 1H, N^+H), 7.54 (dd, 6.9, 1.9, 1H, H-13), 7.45
(m, 1H, H-9), 6.94-7.12 (m, 3H, H3-5), 7.01 (t,6.9,1H, H-12), 4.28 (m, 2H, H-14), 3.41 (m, 2H, H-15), 3.26 (m, 4H, H-16), 2.24 (s, 6H, H-18), 1.50 (t, 7.4, 6H, H-17)

^{13}C-NMR ($CDCl_3$, δ ppm, J Hz): 168.5 (C7), 160.7 (d, 264.3, C11), 140.0 (C1), 134.9 (C2,6), 134.8 (d, 9.4, C-13), 131.5 (d, 4.0, C8), 130.6 (C3,5), 129.2 (C4), 128.8 (d, 2.6, C9), 122.6 (q, 273.1, CF_3), 119.4 (dq, 33.5, 12.8, C10), 117.3 (d, 21.2, C12), 47.7 (C15), 47.2 (C16), 45.3 (C14), 18.7(C18), 8.6 (C17)

FT-IR (solid in ATR, υ cm^{-1}): 2979.5, 2360.4, 2342.1, 1644, 1607.4, 1581.3, 1540.9, 1507.1, 1457, 1381.8, 1331.6, 1171.5, 1114.7, 1055.8, 899.6, 860.1, 846.6, 809, 775.2, 764.6, 683.6

In table II there are presented the results of qualitative screening, and in table III are presented the MIC values for the compounds **6a-f** on the tested bacterial strains. The most of the tested benzamides presented a broad spectrum of activity.

Table II

The results of qualitative screening

Microbial strain	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**
Klebsiella pneumoniae	+	+	+	-	+	+
Escherichia coli	+	+	+	-	+	+
Pseudomonas aeruginosa	+	+	+	-	+	+
Staphylococcus aureus	+	+	+	+	+	+
Enterococcus faecalis	+	+	+	-	+	+

Table III

In vitro antimicrobial activity expressed as MIC (μg/ mL)

Microbial strain	**6a**	**6b**	**6c**	**6d**	**6e**	***6f***
Klebsiella pneumoniae	31.25	31.25	3.9	>1000	7.8	*15.6*
Escherichia coli	500	7.8	125	>1000	62.5	*31.25*
Pseudomonas aeruginosa	250	7.8	250	>1000	250	*250*
Staphylococcus aureus	62.5	125	125	250	125	*62.5*
Enterococcus faecalis	*250*	*250*	*125*	*>1000*	*62.5*	*31.25*

Conclusions

This paper presents the synthesis of new benzanilides, derived from 2,6-dimethylaniline. The new compounds were tested for their *in vitro* antibacterial activity expressed as MIC (μg/mL). The *in vitro* antimicrobial activity depends on the substituents in the benzoyl ring and the presence of fluoro- and trifluoromethyl substituents is correlated in our studies with an optimal antimicrobial activity. This substitution also seems to be optimal for high activity against *Staphylococcus aureus,* suggesting a possible use of these compounds in treatment of MRSA (methicillin-resistant *Staphylococcus aureus*) infectious diseases.

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