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Original article

Synthesis and antimicrobial activities of 5-amidobenzotriazole derivatives

G Caliendo[1], E Novellino[1], G Sagliocco[2], V Santagada[1], C Silipo[1]*, A Vittoria[1]*

[1]*Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli, Via D Montesano 49, 80131 Naples;*
[2]*Istituto di Igiene e Medicina Preventiva, II Facoltà di Medicina e Chirurgia, Via Pansini, 80131 Naples, Italy*

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Summary — A series of 2-cyclopentyl-5-amidobenzotriazole derivatives has been prepared and characterized. Shake-flask partition coefficients (log P) and capacity factors (log k') were experimentally determined and correlated through regression analysis. The *in vitro* antimicrobial activity of prepared compounds against gram-positive, gram-negative bacteria and *Candida albicans* are discussed in function of their structural features. Some derivatives are characterized by interesting antimicrobial activities.

Résumé — Une série de dérivés du 2-cyclopentyl-5-amidobenzotriazole a été préparée et caractérisée. *Les coefficients de partage (log P) et les facteurs de capacité (log k') ont été déterminés expérimentalement et corrélés par analyse de régression. L'activité antimicrobienne* in vitro *de ces composés vis-à-vis des bactéries gram-positif, gram-négatif ainsi que* Candida albicans *a été discutée en fonction de leurs caractéristiques structurales. Quelques dérivés se distinguent par des activités antimicrobiennes intéressantes.*

benzotriazoles / lipophilicity / antimicrobial activity

Introduction

Benzotriazole derivatives are among the various heterocycles that have received a great deal of attention during the last years; however, the antimicrobial properties of these compounds have been reported almost exclusively in the patent literature. Recently however, only few papers report on compounds such as benzotriazol-1-yl ethanol [1] and 1-arylaminomethylbenzotriazole [2] an antimicrobial activity.

In previous papers [3, 4] we have been interested in this class of compounds and have carried out structural modifications in an attempt to obtain compounds showing auxine-like activity and offering adequate variation in lipophilic character. Among the compounds studied, 2-cyclopentyl-5-nitrobenzotriazole (**1c**) was shown to possess weak activity against *S aureus* ; moreover, this compound showed an octanol / water partition coefficient (log P) of 3.10, which easily allows a variation of lipophilic properties in a wide range by introducing adequate substituents on the aromatic moiety of the benzotriazole nucleus.

Beginning with these observations and, with the aim of bringing out possible potentiation of antimicrobial activity, we have planned out the preparation of structurally related benzotriazole derivatives which are reported in table I. Substituents characterized by different chemical and physical properties have been selected in order to obtain the maximum amount of information with the lowest number of compounds.

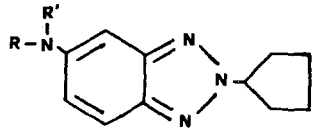
Chemistry

Compounds of general formula reported in table I were synthesized, according to scheme 1, by reaction of the 2-cyclopentyl-5-aminobenzotriazole (**2**) with the appropriate acidic chloride.

The 5-aminobenzotriazole derivatives were prepared by catalitic reduction of the corresponding 5-nitro-derivatives obtained by reaction of 5-nitrobenzotriazole with cyclopentylbromide. This reaction, carried in absolute ethanol and sodium ethoxide, gave a mixture of products **1a–c** corresponding to 1,5-, 1,6- and 2,5-substituted isomers, respectively, whose separation was performed by chromatography on silica gel column using CH_2Cl_2–hexane 9:1 v / v as eluent. This reaction gave a mixture of the three isomers in 75% yield; the compound bearing the substituent in position 2 was obtained in higher yield.

*Correspondence and reprints

Table I. Physicochemical properties of 2-cyclopentyl-5-amidobenzotriazole derivatives **3–21**.



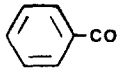
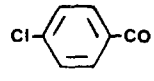
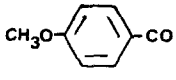
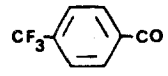
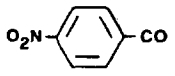
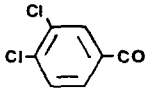
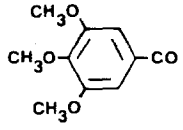
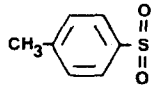
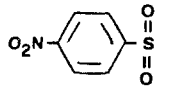
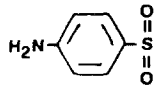
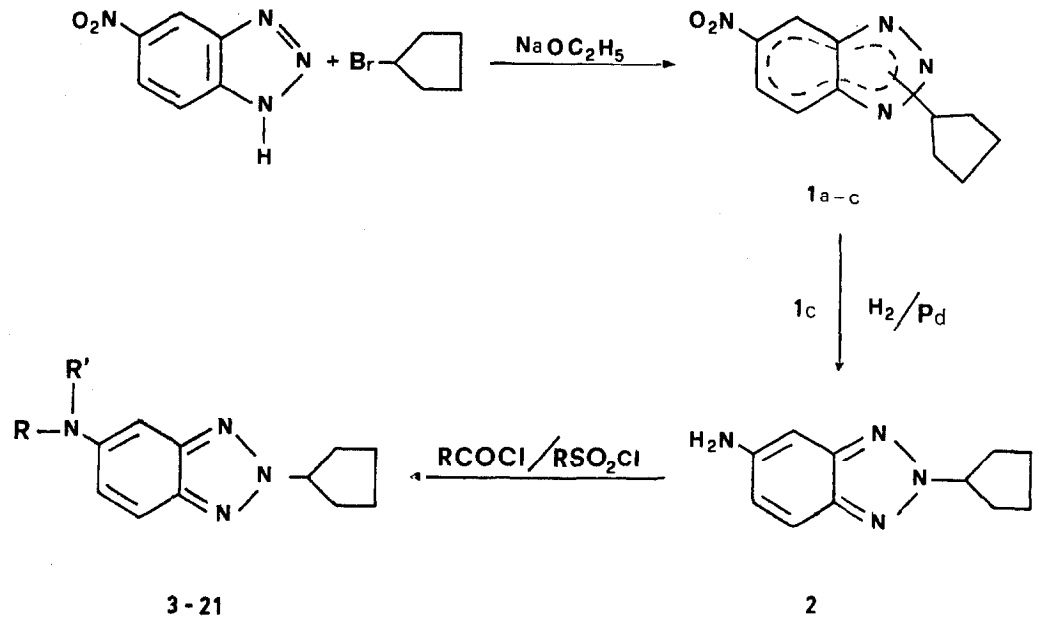
N	R	R'	Molecular formula	M.W.	m.p.	yield %	log P[a]	log k'
3	CH_3CO	H	$C_{13}H_{16}N_4O$	244.30	93–95	33	2.45 (± 0.02)	0.28
4	$ClCH_2CO$	H	$C_{13}H_{15}N_4OCl$	278.74	150–151	40	2.78 (±0.01)	0.43
5	Cl_2CHCO	H	$C_{13}H_{14}N_4OCl_2$	313.19	118–120	24	3.40[b]	0.68
6	Cl_3CCO	H	$C_{13}H_{13}N_4OCl_3$	347.63	135–137	34	4.12 (±0.04)	1.03
7	$(CH_3)_3CCO$	H	$C_{16}H_{22}N_4O$	286.38	135–136	49	3.40[b]	0.68
8	CH_3CO	CH_3CO	$C_{15}H_{18}N_4O_2$	286.33	95–97	32	2.73[b]	0.36
9	C_2H_5OCO	H	$C_{14}H_{18}N_4O_2$	274.32	121–122	49	3.43 (±0.02)	0.69
10	$(CH_3)_2CHCH_2OCO$	H	$C_{16}H_{22}N_4O_2$	302.38	120–122	48	4.28 (±0.04)	1.14
11		H	$C_{18}H_{18}N_4O$	306.37	164–166	39	3.85 (±0.03)	0.81
12		H	$C_{18}H_{17}N_4OCl$	340.81	179–181	29	4.11[b]	1.16
13		H	$C_{19}H_{20}N_4O_2$	336.39	179–180	60	4.03 (±0.04)	0.92

Table I. *Continued.*

14		H	$C_{19}H_{17}N_4OF_3$	374.37	215–216	36	4.95 (±0.05)	1.34
15		H	$C_{18}H_{17}N_5O_3$	351.37	227–228	32	3.75 (±0.02)	0.99
16		H	$C_{18}H_{16}N_4OCl_2$	375.26	206–208	50	5.32[b]	1.59
17		H	$C_{21}H_{24}N_4O_4$	396.45	128–130	40	3.87[b]	0.90
18		H	$C_{12}H_{16}N_4O_2S$	280.35	126–127	38	2.44 (±0.01)	0.20
19		H	$C_{18}H_{20}N_4O_2S$	356.45	125–127	31	3.91[b]	0.91
20		H	$C_{17}H_{17}N_5O_4S$	387.42	193–195	36	3.82[b]	0.88
21		H	$C_{17}H_{19}N_5O_2S$	357.44	206–208	65	2.48 (±0.02)	0.18

[a]Number in parentheses indicates the 95 per cent confidence interval.
[b]These values are calculated from equation 1.



Scheme 1.

All compounds listed in table I were characterized by PMR, IR and UV spectroscopy.

Examination of IR spectra shows the presence of characteristic absorption frequencies reflecting the nature of the functional group and the substitution on position 2 at the benzotriazole ring. The nature of the functional group was evidenced by the presence of stretchings at 3220–3350 cm^{-1} (NH) and 1680–1650 cm^{-1} (C=O) for the amides; at 3430 (NH_2), 1680 (C=O) and 1340 (SO_2) cm^{-1} for the sulfonamido derivatives, while the compounds bearing the NO_2 group were characterized by a sharp stretching at 1530 cm^{-1}.

The ultraviolet spectra show the presence of an aromatic chromophoric system for all compounds. Particularly the aliphatic amides **3, 7** and **8** show a single absorption maximum ranging between 280 and 300 nm, while the sulfonamido derivatives **18–21** show an intense absorption maximum between 270–292 nm. The UV spectra of arylamido-derivatives **11–17** were characterized by 2 absorption maxima ranging between 258–263 nm and 300–306 nm; while the chloroaliphatic amides **4–6** show absorption maxima ranging between 233–246 nm and 295–303 nm. In addition to IR and UV spectra, all compounds reported in this paper were characterized by PMR spectroscopy whose data were fully consistent with the described structures.

Finally, partition coefficients (log P) were determined in octanol / water system and the capacity factors (log k') of all substances considered were correlated, through regression analysis, with the corresponding partition coefficients.

Results and Discussion

It is well known that a large number of organic molecules exert their antimicrobial effects by so-called non specific interactions which can be accounted for by partition coefficient (log P). Moreover, the lipophilic / hydrophilic balance of a drug is a major factor in determining its passage across membranes within the body for absorption, tissue penetration and elimination.

Since large electronic and steric differences [4, 5] occur in the two heteroring systems of 1- and 2-substituted isomers, only the 2,5-substituted derivatives were considered in this report in order to obtain a better evaluation of lipophilic influence on antimicrobial activities. In fact, the preparation of this set of derivatives has allowed us to vary the lipophilic character of the substituents in position 5 without seriously affecting the electronic delocalization of the benzotriazole nucleus. This fact is particularly interesting since there are numerous variables able to alter cellular penetration and the manifestation of activity within the same cell.

The standard method for determining the partition coefficient of a drug is to partition it between octanol and water phases, then measure the concentration of drug in both. If the classical 'shake-flask' technique is employed, this approach suffers from the disadvantage that quantitative measurements of very small amounts of drug end up in the hydrophilic phase in the range of high log P values. When it is desired to measure the partition coefficients of a number of closely related drugs, as are those listed in table I, an alternative

method is a reverse phase HPLC method whose usefulness is well established; in addition, this method allows solubility problems to be overcome. Mixtures of water and methanol are shown to be best in order to obtain good separation with reasonable retention times. Data reported in table I are relative to a methanol / water mixture in the ratio 3:2 (v / v). The chromatographic indices (log k') were correlated with the corresponding measured octanol / water partition coefficients (compounds **3, 4, 6, 9, 10, 11, 13, 14, 15, 18** and **21**) and equation 1 was used to estimate the remaining values.

$$\log P = 2.10(\pm 0.30); \log k' + 1.98(\pm 0.25) \quad (1)$$
$$n = 10;\ r = 0.985;\ s = 0.163;\ F_{1,8} = 268.5$$

In this equation, n is the number of compounds considered, r is the correlation coefficient, s the standard deviation of the regression and the figures in parentheses are the 95% confidence limits; F represents the statistical significance of the equation. The good correlation coefficient and the low standard deviation of equation (1), based on data covering a wide range of log P values (from 2.44 to 5.32), clearly demonstrate an excellent HPLC modeling of the 'shake-flask' procedure.

Table II describes the *in vitro* antimicrobial activities of title compounds against Gram-positive *(Staphylococcus aureus)* and Gram-negative bacteria *(Salmonella wiener, Serratia marcescens* and *Pseudo-*

Table II. Antimicrobial activity of compounds **3–21** and some reference antibiotics tested in the same conditions (MIC μg / ml, in parentheses log 1 / C).

Compound	*S. aureus*	*P. aeruginosa*	*S. wiener*	*S. marcescens*	*C. albicans*
3	100 (3.39)	>100 (<3.39)	100 (3.39)	100 (3.39)	>100 (<3.39)
4	100 (3.44)	100 (3.44)	100 (3.44)	100 (3.44)	50 (3.75)
5	50 (3.80)	100 (3.50)	100 (3.50)	100 (3.50)	>100 (<3.50)
6	50 (3.84)	>100 (<3.54)	>100 (<3.54)	>100 (<3.54)	100 (3.54)
7	100 (3.46)	100 (3.46)	100 (3.46)	100 (3.46)	>100 (<3.46)
8	>100 (<3.46)	>100 (<3.46)	>100 (<3.46)	>100 (<3.46)	>100 (<3.46)
9	100 (3.44)	100 (3.44)	100 (3.44)	100 (3.44)	50 (3.74)
10	100 (3.48)	100 (3.48)	100 (3.48)	100 (3.48)	100 (3.48)
11	100 (3.48)	100 (3.48)	100 (3.48)	100 (3.48)	>100 (<3.48)
12	100 (3.53)	100 (3.53)	100 (3.53)	100 (3.53)	100 (3.53)
13	100 (3.53)	>100 (<3.53)	100 (3.53)	>100 (<3.53)	>100 (<3.53)
14	>100 (<3.57)	>100 (<3.57)	>100 (<3.57)	>100 (<3.57)	>100 (<3.57)
15	100 (3.54)	>100 (<3.54)	100 (3.54)	100 (3.54)	100 (3.54)

Table II. *Continued.*

16	>100 (<3.57)	>100 (<3.57)	>100 (<3.57)	>100 (<3.57)	>100 (<3.57)
17	100 (3.60)	100 (3.60)	100 (3.60)	100 (3.60)	100 (3.60)
18	>100 (<3.45)	>100 (<3.45)	>100 (<3.45)	>100 (<3.45)	50 (3.75)
19	100 (3.55)	>100 (<3.55)	100 (3.55)	100 (3.55)	>100 (<3.55)
20	100 (3.59)	100 (3.59)	100 (3.59)	100 (3.59)	25 (4.19)
21	>100 (<3.55)	>100 (<3.55)	>100 (<3.55)	>100 (<3.55)	>100 (<3.55)
Ampicillin	12.5 (4.45)	>100 (<3.54)	25 (4.15)	>100 (<3.54)	--
Cephalothin	12.5 (4.50)	>100 (<3.60)	100 (3.60)	>100 (<3.60)	--
Erythromycin*	12.5 (4.77)	>100 (<3.87)	>100 (<3.87)	>100 (<3.87)	--
Gentamicin**	25 (4.43)	12.5 (4.73)	12.5 (4.73)	12.5 (4.73)	--
Amphotericin B	--	--	--	--	12.5 (4.87)

(*) Calculated as erythromycin A ($C_{37}H_{67}NO_{13}$, mw 733.9).
(**) Calculated as gentamicin C_1 ($C_{21}H_{43}N_5O_7 \cdot 2H_2SO_4$, mw 673.6).

monas aeruginosa), as well as their fungistatic activity against *Candida albicans*; moreover, table II includes the antimicrobial activities of some reference antibiotics tested in the same conditions. As mentioned above, it is interesting to point out that 2-cyclopentyl-5-nitrobenzotriazole exhibited only weak activity against *S aureus*, while the introduction of the amide group in position 5 resulted in gain of activity in several instances. Actually, compounds listed in table I may be classified as alyphatic (**3–8**) and aromatic (**11–17**) amides, carbamates (**9, 10**) and sulfonamides (**18–21**).

The data, expressed as log 1/C, indicate the following general trends in structure-activity relationships: although the compounds are characterized by a wide variation in their lipophilic properties, they display only narrow ranges of inhibitory effects against all of the tested strains; the poor biological variation does not allow any statistically significative consideration from the quantitative point of view. However, considering the alyphatic amides, when the hydrogens of the methyl group are stepwise replaced by chlorine atoms an increase of *in vitro* activity, especially *versus S aureus*, was observed. Against Gram-negative organisms, these compounds have activity in the same range as against *S aureus*, but there is a loss of activity for the most lipophilic compound **6**, that is, while a further increase in lipophilicity gave higher activity against *Staphylococcus aureus*, the reverse seems true for the Gram-negative organisms. Note that the monochloro-substitution led to a compound **4**, active not only against all tested bacterial strains, but also versus *Candida albicans*; moreover, if the remaining hydrogen atom of the amide group is replaced by a second acetyl group, as in compound **8**, there is a complete loss of activity. In the two carbamate derivatives **9** and **10**, again the lipophilicity seems to increase antibacterial activity, while the reverse is observed for their antifungal action.

A general trend to decreased Gram-negative activity, as lipophilicity increases, can be seen by examination of data relative to benzoyl amides. In the

extremely lipophilic compounds **14** (log P = 4.95) and **16** (log P = 5.32), not only Gram-negative activity, but also Gram-positive action is completely lost. The nature of the substituent on the phenyl ring seems irrelevant for antimicrobial action; in fact, the activity profile of the unsubstituted benzoyl derivative is similar to that of compounds **12, 15** and **17** which show comparable lipophilicities.

In general, the introduction of the sulfonamide group in exchange for the carboamide group resulted in loss of antibacterial activity, with the exception of compound **20** which displayed moderate potency against Gram-positive and Gram-negative organisms; concerning the influence of chemical modification on the antifungal activity, the *p*-nitrobenzenesulfonamido group seems to be the best substituent in all set of derivatives considered in table II.

This class of benzotriazole derivatives deserves further investigation; however, any general hypothesis regarding their mechanism of action must include the effects of hydrophobic-lipophilic interactions while one cannot make any firm statement about the nature of interactions involved in their antifungal properties.

Synthesis and evaluation of further series of derivatives, with varied substituents at the 2- and 5-position of benzotriazole nucleus, will be reported in another paper.

Experimental protocols

All compounds gave satisfactory elemental analyses (C, H, N and S) within ±0.3% of the theoretical values and were characterized by IR, UV and PMR spectroscopy. Melting points, determined with a Kofler apparatus, are uncorrected.

Nuclear magnetic resonance (PMR) spectra were recorded on a Bruker WM 250 spectrometer. Chemical shifts are reported in parts per million (δ) from an internal tetramethylsilane standard. Splitting patterns are designed as follows: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. Infrared (IR) spectra were obtained on a Perkin–Elmer Model 177 spectrophotometer.

Although the IR and PMR spectra data of compounds **3–21** are not included because of the lack of unusual features, they were obtained for all compounds reported and were consistent with the assigned structures.

UV spectra were taken on a Beckman DU–40 spectrophotometer. Chromatographic separations were performed on a silica gel column (Kieselgel 40, 0.063–0.200 nm, Merck). Analytical thin-layer chromatography (TLC) was carried on Merck silica gel–60 F–254 glass-backed plates and visualized by UV.

The partition coefficients (*P*) were determined according to the classic 'shake-flask' procedure [6] at room temperature, using octanol as lipophilic phase and water as hydrophilic phase. Octanol and water phases were adjusted in volume so that satisfactory amounts of sample ended up in each phase. The analysis of the concentrations of the partitioned substance were made using a Beckman DU–40 spectrophotometer. Four determinations were made on each tested compound.

High performance liquid chromatography was performed using a μ-Bonda-pack C_{18} stainless steel column (3.9 mm x 100 mm) from Waters Associates. Methanol / water 3:2 (v / v) at a constant flow rate of 0.5 ml / min was employed as eluent. Elution was continued until a stable base line was obtained. Solutions were first filtered (Millipore) and degassed to reduce contamination or column clogging. Compound retentions were expressed by the logarithm of the capacity factor (k'), defined as $\log k' = \log [(t_r - t_o)/t_o]$, where t_r and t_o are the elution times of the retained and unretained compound, respectively. Acetone served as a suitable non-retained compound to define the dead volume t_o.

*Preparation of 2-cyclopentyl-5-nitrobenzotriazole (**1c**)*

In a general typical procedure, clean sodium (2.4 g, 0.1 mol) was added to anhydrous ice-cooled ethanol (100 ml), and vigorously stirred until it was completely dissolved. Added in succession to this solution was 5-nitrobenzotriazole (11.9 g, 0.1 mol) and then cyclopentylbromide (14.9 g, 0.1 mol). The reaction mixture was kept under reflux for 24 h and monitored by TLC (CH_2Cl_2 / hexane 9:1 v / v).

After cooling, the ethanol was removed under reduced pressure and the residue was treated with diethyl ether (100 ml), separated from the sodium bromide and washed with 2N sodium hydroxide and water to remove the unreacted 5-nitrobenzotriazole. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. TLC examination of the reaction mixture showed the formation of 3 UV absorbing products, one of which was preponderant. Fractionation, performed on a silica gel column (5 x 80 cm), using as eluent CH_2Cl_2 / hexane 9:1 v / v, led to isolation of the more fast moving compound which, on the basis of UV and PMR spectra analysis, resulted in the 2-cyclopentyl-5-nitrobenzotriazole. Further elution led to isolation of the 2 remaining isomers.

Analytical purification of each product was obtained by crystallization from the appropriate solvent.

Relevant data are: $C_{11}H_{12}N_4O_2$, mw 232.24; mp 87–88°C (diethyl ether), yield 38%; IR ($CHCl_3$) υ_{max} 1530 cm^{-1}; UV (EtOH) λ_{max} 291 and 248 nm (log ε = 4.08 and 4.27). PMR ($CDCl_3$) δ: 8.85 (1H, dd, J = 1.1 Hz, H–4); 8.22 (1H, dd, J = 8.5 and 1.1 Hz, H–6); 7.95 (1H, d, J = 8.5 Hz, H–7); 5.40 (1H, cm, H–1'); 2.4 (4H, cm, H–2' and H–5'); 2.1–1.7 (4H, cm, H–3' and H–4').

*Preparation of 2-cyclopentyl-5-aminobenzotriazole (**2**)*

To 0.25 mol of 2-cyclopentyl-5-nitrobenzotriazole in dry methanol (50 ml), 1.5 g of 10% palladium on activated carbon was added and the resulting mixture was stirred at room temperature under hydrogen pressure until the theoretical amount of hydrogen was absorbed. TLC examination (diethyl ether) showed the quantitative formation of the 2-cyclo-pentyl-5-aminobenzotriazole. The suspension was then filtered and the solid phase washed twice with dry methanol. The filtrate, evaporated to dryness *in vacuo*, gave the expected 2-cyclopentyl-5-aminobenzotriazole (**2**) as pale yellow oil, successively characterized by IR and PMR spectroscopy: $C_{11}H_{14}N_4$, mw 202.26; oil, IR ($CHCl_3$) υ_{max} 3500 cm^{-1}; PMR ($CDCl_3$) δ: 7.95 (1H, d, J = 8.5 Hz, H–7); 7.80 (1H, d, J = 1.1 Hz, H–4); 7.37 (1H, dd, J = 8.5 and 1.1 Hz, H–6); 5.35 (1H, cm, H–1'); 2.4 (4H, cm, H–2' and H–5'); 2.1–1.7 (4H, cm, H–3' and H–4').

Preparation of 2-cyclopentyl-5-amidobenzotriazole derivatives (3–20)

In general typical procedure, 0.01 mol of 2-cyclopentyl-5-aminobenzotriazole (**2**) was dissolved in 50 ml of anhydrous benzene. Added successively to this solution was 0.01 mol of the appropriate acidic chloride and 0.01 mole of anhydrous pyridine. The reaction mixture was vigorously stirred and refluxed for 4 h, then cooled and filtered; the solid salt that formed was washed twice with dry ether. The combined ether solution was evaporated and distilled to give compounds **3–20**, which were further purified by crystallization from diethyl ether or chloroform. Compound **8** was obtained by reaction of **2** with two equivalents of acetyl chloride. Yields are reported in table I.

*Preparation of 2-cyclopentyl-5-(*p*-aminobenzensulfonamido)-benzotriazole (21)*

2.0 g (0.01 mol) of 2-cyclopentyl-5-aminobenzotriazole (**2**) were dissolved in 35 ml of dry pyridine. To this solution, cooled in ice bath and stirred, were added dropwise 2.41 g (0.01 mol) of *p*-acetamido-benzenesulfochloride. The reaction mixture was allowed to stand 20 h at room temperature and successively heated at 60°C for an additional 8 h. After this time, the reaction mixture was distilled *in vacuo* until *ca* 25 ml of pyridine were removed and the residue was poured into ice water (70 g). The solid formed was separated, washed twice with water and dried.

The 2-cyclopentyl-5-(*p*-acetamidobenzensulfonamido)benzotriazole formed (72% yield) was crystallized from methanol mp 227–229°C; $C_{19}H_{21}N_5SO_3$ mw 399.47; IR ($CHCl_3$) υ_{max} 3430, 3340, 1680, 1340 and 1140 cm^{-1}; PMR ($CDCl_3$) δ: 7.72 (1H, d, J = 8.5 Hz, H–7); 7.50 (1H, d, J = 1.1 Hz, H–4); 7.3 (1H, bs, NH); 7.10 (1H, dd, J = 8.5 and 1.1 Hz, H–6); 7.55 (2H, d, J = 8.5 Hz, H–2' and H–6'); 6.58 (2H, d, J = 8.5 Hz, H–3' and H–5'); 6.5 (1H, bs, NH); 5.27 (1H, cm, CH); 2.30 (4H, cm, CH_2-CH-CH_2); 2.20 (3H, s, CH_3); 2.0 and 1.8 (4H, m, remaining aliphatic protons).

The hydrolysis of the acetyl group was performed by refluxing in NaOH 2 N for 1 h. After cooling, the pH of the solution was adjusted to 6 with HCl 2 N and the precipitate formed was separated, washed with water and dried. Crystallization from ethanol led to pure 2-cyclopentyl-5-(*p*-aminobenzensulfonamido)benzotriazole (**21**) in 90% yield.

Antimicrobial activity

Antimicrobial activity of the prepared compounds was tested against Gram-positive and Gram-negative bacteria, as well as against fungi.

Minimum inhibitory concentrations (MICs) were determined by the broth dilution technique [7]. Tested microorganisms were *Staphylococcus aureus* (ATCC 6538); *Serratia marcescens* and *Salmonella wiener* (clinical isolates); *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231). The substances, dissolved in 10% acetone-water solution at 2 mg / ml concentration, were diluted in broth in the range 200–12.5 μg / ml.

In the testing antibacterial procedure, Mueller-Hintor broth (BBL) was used as a growth medium [8]; cultures grown at 37°C overnight were diluted in the same broth, approximately 10^7 CFU / ml, and used as inoculum. Test cultures were incubated at 37°C for 24 h.

In the antifungal testing procedure, Sabouraud dextrose broth (BBL) was employed and the inoculum was represented from 48 h growth of the organism at 25°C; test tubes were also incubated at 25°C for 48 h.

All the results are presented as μg / ml or as log 1 / C, where C represents the molar concentration; the lowest concentration of antimicrobial agent that results in the complete inhibition of visible growth of microorganisms represents the minimal inhibitory concentration (MIC) (table II).

Some very active antimicrobial substances were employed during the test procedures as references. They were gentamicin sulfate (Gram–); erythromycin (Gram+); ampicillin and cephalothin (Gram+ and Gram–); amphotericin B (fungi). The obtained MICs are listed in table II.

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References

1 Negoro K, Takemoto T, Ozaki H, Oka S (1977) *Hiroshima Daigoku Kobakubu Kenkyu Hokoku* 26, 9
2 Hussain S, Murtaza N, Qureschi IH (1987) *Pak J Sci Ind Res* 30, 97
3 Sparatore F, La Rotonda MI, Caliendo G, Novellino E, Silipo C, Vittoria A (1983) *Il Farmaco Ed Sci* 43, 141
4 Sparatore F, La Rotonda MI, Caliendo G, Novellino E, Silipo C, Vittoria A (1988) *Quant Struct Act Relat* 7, 119
5 Giordano F, Zagari A (1978) *J Chem Soc Perkin II*, 312
6 Leo A, Hansch C, Elkins D (1971) *Chem Rev* 71, 525
7 Barry AL (1976) *In: The Antimicrobial Susceptibility Test: Principles and Practices*. Lee & Febiger, Philadelphia
8 World Health Organization (1961) *In: Standardization of Methods for Conducting Microbic Sensitivity Test*. Technical reports series No 210, WHO, Geneva