SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW RUTIN SEMISYNTHETIC DERIVATIVES AS ANTIBACTERIAL AGENTS

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Abstract

Many studies show that flavonoids have a lot of biological properties, including the antimicrobial effect. It is also known that rutin is able to increase the antibacterial activity of other compounds. On the other hand, the antibacterial sulphonamides have weak water solubility, which is responsible for the renal serious secondary effects. Starting from these facts, we synthesised some water soluble rutin derivatives with antibacterial sulphonamides, treating rutin with 1,3-dichloro-2-propanol, 1-bromo-3-chloro-propane, 2-dibromethane and dibrommethane, and then with sulfadimethoxyne, sulfamethoxazole, sulfachloropyridazine, sulfapyridine, sulfisoxazole, sulfacetamide, sulfadiazine, sulfamerazine, sulfathiazole. Molecular formula, weight, yield, melting points and solubility have characterized the new derivatives. Elemental analysis and spectral analysis (UV and IR) have confirmed the structure of the new compounds. *In vitro* and *in vivo* microbiological assays were performed. Some of them showed marked antibacterial properties. The most active compounds are the sulfapyridine and sulfachloropyridazine derivatives, that have an equal activity or are even more active than cotrimoxazole.

Rezumat

Numeroase studii au stabilit faptul că flavonoidele prezintă proprietăţi biologice diverse, inclusiv efect antibacterian. De asemenea, există dovezi că rutozidul potenţează acţiunea antimicrobiană a unor compuşi cunoscuţi pentru aceste proprietăţi. Pe de altă parte, sulfonamidele antibacteriene prezintă o slabă solubilitate în apă, caracteristică responsabilă de efectele adverse la nivel renal. Pornind de la aceste premize, am sintetizat o serie de derivaţi hidrosolubili ai rutozidului cu sulfamide antibacteriene, prin tratarea rutozidului cu 1,3-dicloro-2-propanol, 1-brom-3-cloro-propan, 2-dibrom-etan şi dibrom-metan, în mediu de metoxid de sodiu, şi apoi cu sulfadimetoxina, sulfametoxazol, sulfacloropiridazina, sulfapiridina, sulfizoxazol, sulfacetamida, sulfadiazina, sulfamerazina, sulfatiazol. Noii derivaţi au fost caracterizaţi prin formula şi masa moleculară, randament, punct de topire şi solubilitate, iar structura a fost confirmată prin analiză elementală şi spectrală (în UV şi IR). Au fost efectuate teste microbiologice *in vitro* şi *in vivo*. Unii dintre compuşi prezintă bune proprietăţi antibacteriene; cei mai activi compuşi sunt derivaţii sulfapiridinei ş i sulfacloropiridazinei, care prezintă o activitate antibacteriană egală sau chiar mai pronunţată decât cea a cotrimoxazolului.

Keywords: flavonoids, rutin, sulphonamide, antibacterial activity

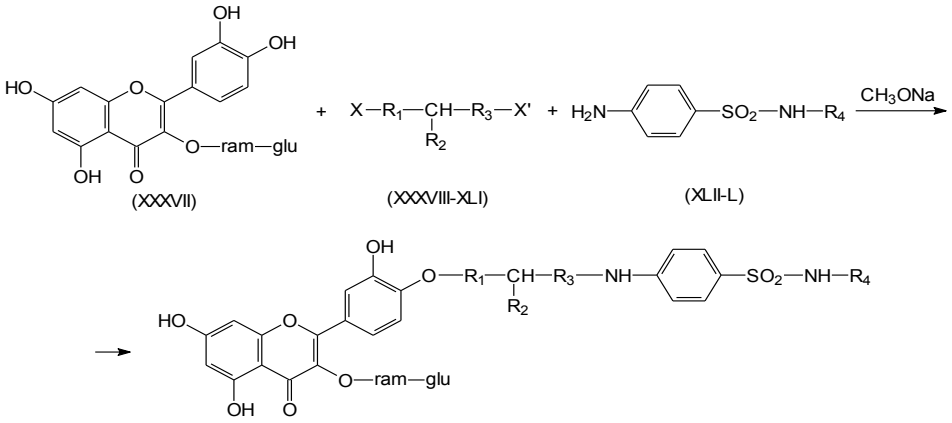
Introduction

Many studies showed that flavonoids have a lot of biological properties (antioxidant, anti-inflammatory, antiallergenic, antimicrobial, antiviral, antitumor actions) through various action mechanisms and in many cases, are capable to protect against chronic and degenerative diseases (like cancer, cardiovascular and immune system disorders) [1,4,6-8,10]. It is also known that rutin is able to increase the antibacterial activity of other compounds. On the other hand, the antibacterial sulphonamides have weak water solubility, a fact that is responsible for the serious secondary effects (especially on the renal level). To continue our research [5,9] and starting from the above facts, we synthesised some water soluble rutin derivatives with antibacterial sulphonamides and we performed microbiological assays.

Materials and Methods

All commercial chemicals and solvents are reagent grade and were used without further purification. Rutin (97-102%) was purchased from Across Organics; solvents (methanol, isopropanol, ethanol) and sulphonamides were purchased from Sigma-Aldrich; halogenated reagents (1, 3-dichloro-2-propanol; 1-brom-3-chloro-propane; 1, 2-dibrom-ethane and dibrommethane) were purchased from Merck-Schuchardt, sodium was delivered by Riedel-de-Haen AG and Silicagel was purchased from Fluka. Mueller Hinton Agar was delivered by Blulux Laboratories Ltd. Bacterial strains were obtained from the Microbiology Department collection. Melting points are uncorrected and were measured in open capillary tubes on an Electrothermal Mel-Temp device; the elemental analysis was performed on an "Exeter Analytical" CE-440 elemental analyser. The IR spectra were recorded on a FT/IR "Jasco 670 Plus" spectrometer. The UV spectra were recorded on a "Hewlett-Packard" 8453E UV-VIS spectrometer. The general procedure for the new compounds synthesis was as follows: 6.64 g of rutin (compound number XXXVII – Figure 1) (0.01 mol) was dissolved in 180 mL sodium methoxide (containing 0.23 g sodium), reflux heated for 30 minutes, subsequently treated with 0.01 mol of 1, 3-dichloro-2-propanole; 1-brom-3-chloro-propane; 1, 2-dibrom-ethane and dibrommethane (compound number XXXVIII-XLI – Figure 1) and finally, reflux heated for six hours with the XLII-L sulphonamides obtaining the corresponding compounds I-XXXVI. From the reaction mixtures, which are yellow-orange solutions, we obtained the crude derivatives through precipitation with isopropanol, filtration and 37° temperature drying.

The crude compounds were purified on the column chromatography (0.5 cm x 15 cm; Silicagel H (10-40 μm), elution with 50^{c} alcohol: 0.25-0.30 ml/min.) (figure 1).



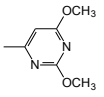
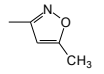
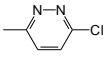
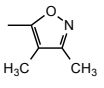
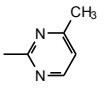
Comp. No.	X	X'	R_1	R_2	R_3	R_4
I	Cl	Cl	CH_2	OH	CH_2	
II	Br	Cl	CH_2	H	CH_2	
III	Br	Br	CH_2	H	-	
IV	Br	Br	-	H	-	
V	Cl	Cl	CH_2	OH	CH_2	
VI	Br	Cl	CH_2	H	CH_2	
VII	Br	Br	CH_2	H	-	
VIII	Br	Br	-	H	-	
IX	Cl	Cl	CH_2	OH	CH_2	
X	Br	Cl	CH_2	H	CH_2	
XI	Br	Br	CH_2	H	-	
XII	Br	Br	-	H	-	
XIII	Cl	Cl	CH_2	OH	CH_2	
XIV	Br	Cl	CH_2	H	CH_2	
XV	Br	Br	CH_2	H	-	
XVI	Br	Br	-	H	-	
XVII	Cl	Cl	CH_2	OH	CH_2	
XVIII	Br	Cl	CH_2	H	CH_2	
XIX	Br	Br	CH_2	H	-	
XX	Br	Br	-	H	-	
XXI	Cl	Cl	CH_2	OH	CH_2	—CO—CH_3
XXII	Br	Cl	CH_2	H	CH_2	
XXIII	Br	Br	CH_2	H	-	
XXIV	Br	Br	-	H	-	
XXV	Cl	Cl	CH_2	OH	CH_2	
XXVI	Br	Cl	CH_2	H	CH_2	
XXVII	Br	Br	CH_2	H	-	
XXVIII	Br	Br	-	H	-	
XXIX	Cl	Cl	CH_2	OH	CH_2	
XXX	Br	Cl	CH_2	H	CH_2	
XXXI	Br	Br	CH_2	H	-	
XXXII	Br	Br	-	H	-	
XXXIII	Cl	Cl	CH_2	OH	CH_2	
XXXIV	Br	Cl	CH_2	H	CH_2	
XXXV	Br	Br	CH_2	H	-	
XXXVI	Br	Br	-	H	-	

Figure 1
The synthesis of rutin derivatives noted as I-XXXVI

The antimicrobial activity was determined by the disc diffusion method, using the test organisms as seen in table II. A culture suspension (0.5 McFarland turbidity standard) was mixed with melted Mueller-Hinton (1/10 ratio) and the warm mixture was applied on plates. Filter paper discs impregnated with rutin derivatives were used. The plates were incubated at 37°C for 24 hrs for bacteria and at 24°C for 48 hrs for *Candida* spp. and examined. The diameter of the zones of complete inhibition was measured [2].

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were evaluated using the broth dilution method with an *inoculum* of 10^6 CFU/mL (CFU – Colonies Forming Units). Double dilutions of XI, XII, XIII and XV derivatives were tested. The tubes with diluted antimicrobial agent solution are inoculated with an equal volume of the bacterial suspension (*Staphylococcus aureus* ATCC 25923). The last tube resulting in the complete inhibition of visible growth after 20 hours incubation at 37^0C, represents the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was determined by transferring 0.1 mL from each of the tubes showing no growth on the MIC on the surface of the agar plate. The subcultures were incubated 20 hours and the MBC was read as the least concentration, which produced ≥ 99.9% killing of the bacteria [3,11].

In vivo evaluation of the biological activity was performed using six groups of Swiss mice (≈ 25 g) (six females and six males in every goup), housed on wood chips bedding and having free acces to water. Animals were purchased from rodent farm of Cantacuzino Institute Bucharest.The temperature was between 20°C and 22° and relative humidity was maintained between 35% and 45%; the temperature and humidity were continually checked using a digital hygro-thermometer.

All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, regarding the protection of animals used for experimental and other scientific purposes.

The mice were inoculated intraperitoneally with derivatives XI, XII, XIII and XV, in doses of 1/20 from DL50 (12) for seven days and then it induced an experimental infection with 507 strain of *Klebsiella pneumoniae* and 558 strain of *Streptococcus pneumoniae*. Subsequent, mice were inoculated intraperitoneally with derivatives

XI, XII, XIII and XV, in same doses, for another seven days. From the last day of administration the daily survival rate was evaluated.

Results and Discussion

Thirtysix new rutin derivatives were synthesised with a good yield (83-94%). All these compounds are crystalline, hygroscopic, yellow powders, with no odour and slightly bitter taste, soluble in water, alcohol, dimethylsulfoxide and dimethylformamide and no-soluble in 2-propanol, dioxane, acetone, ether, benzene and chloroform. The physico-chemical characterization is shown in table I. The chemical structures have been proved by C,H,N elemental analysis and UV spectral analysis (table I) and also by FT/IR spectroscopy.

The study of the new rutin derivatives IR spectra revealed characteristic absorptions for rutin moiety between 810-3330 cm^{-1} and also for sulphonamide moiety as following: - between 3250-3492 cm^{-1} - N-H stretch; between 1335-1450 and 1122-1257 cm^{-1} – SO_2 stretch; between 1703-1743 cm^{-1} – pyrimidine ring (for sulfadimethoxine); between 844-854 cm^{-1} – methylisoxazole N-O stretch (for sulfamethoxazole); between 1666-1680 cm^{-1} – C=N stretch (for sulfachloropyridazine); between 1370-1460 cm^{-1} – isoxazole ring (for sulfisoxazole); between 1680-1693 cm^{-1} – CO acetyl stretch (for sulfacetamide); between 1577-1725 cm^{-1} – pyrimidine ring (for sulfadiazine and sulfamerazine); between 1543-1554 cm^{-1} – C=N stretch and between 611-642 cm^{-1} – C-S stretch (both for sulfathiazole).

Most of the synthesized compounds have shown good antibacterial activity against *Staphylococcus aureus, S. aureus methycillin-resistant* and especially against *Sarcina lutea* ATCC 9341 (table II). Among the synthesized structures, compounds V-VII and XI-XII showed a good activity against *Klebsiella pneumoniae pneumoniae 1,* comparable with cotrimoxazole activity (figure 2). Almost all synthesized derivatives have been found to be inactive against *Pseudomonas aeruginosa* and all compounds didn't have antifungal activity. Altogether, the XI, XII, XIII and XV derivatives are the most valuable and these compounds were selected for further assays.

Table I

Rutin derivatives characterization and structure confirmation

Comp. No.	Molecular formula	Molecular mass (g)	Yield (%)	Melting point (°C)	C,H,N, elemental analysis (%; calculated / experimental)	UV spectra (λmax (nm))
I	$C_{42}H_{48}N_4O_{25}S$	976.91	86.72	220-225	51.63; 4.95; 5.73 / 51.59; 4.92; 5.70	264, 354
II	$C_{42}H_{48}N_4O_{20}S$	960.91	85.6	215-219	52.49; 5.03; 5.83 / 52.43; 5.07; 5.81	267, 357
III	$C_{41}H_{46}N_4O_{20}S$	946.89	88.54	223-227	52.00; 4.89; 5.91 / 51.97; 4.92; 5.90	266, 356
IV	$C_{40}H_{44}N_4O_{20}S$	932.86	87.04	218-223	51.50; 4.75; 6.00 / 51.47; 4.71; 6.03	267, 357
V	$C_{40}H_{45}N_3O_{20}S$	919.86	89.22	225-228	52.22; 4.93; 4.56 / 52.18; 4.90; 4.53	265, 356
VI	$C_{40}H_{45}N_3O_{19}S$	903.86	90.11	240-244	53.15; 5.01; 4.64 / 53.19; 5.05; 4.62	264, 356
VII	$C_{39}H_{43}N_3O_{19}S$	889.83	88.98	235-238	52.64; 4.87; 4.72 / 52.69; 4.82; 4.75	264, 358
VIII	$C_{38}H_{41}N_3O_{19}S$	875.81	87	226-229	52.11; 4.71; 4.79 / 52.20; 4.81; 4.71	264, 357
IX	$C_{40}H_{43}N_4O_{19}SCl$	951.30	84.65	250-253	50.50; 4.55; 5.88 / 50.45; 4.61; 5.81	264, 357
X	$C_{40}H_{41}N_4O_{18}SCl$	935.31	83.07	247-249	51.36; 4.63; 5.99 / 51.29; 4.65; 6.02	264, 356
XI	$C_{39}H_{41}N_4O_{18}SCl$	921.28	85	259-262	50.84; 4.48; 6.08 / 50.79; 4.43; 6.11	264, 356
XII	$C_{28}H_{39}N_4O_{18}SCl$	907.25	82.48	255-259	50.30; 4.33; 6.17 / 50.26; 4.29; 6.14	264, 357
XIII	$C_{41}H_{45}N_3O_{19}S$	915.87	93.08	272-275	53.76; 4.95; 4.58 / 53.71; 4.98; 4.56	265, 355
XIV	$C_{41}H_{45}N_3O_{18}S$	899.87	94	268-271	54.72; 5.03; 4.67 / 54.67; 4.98; 4.63	266, 356
XV	$C_{40}H_{43}N_3O_{18}S$	885.85	91.67	262-266	54.23; 4.89; 4.74 / 54.18; 4.83; 4.67	266, 357
XVI	$C_{39}H_{41}N_3O_{18}S$	871.82	92.34	265-267	53.72; 4.74; 4.82 / 53.77; 4.76; 4.80	265, 354
XVII	$C_{41}H_{47}N_3O_{20}S$	933.89	83.89	221-226	52.73; 5.07; 4.50 / 52.78; 5.12; 4.53	264, 354
XVIII	$C_{41}H_{47}N_3O_{19}S$	917.89	86.24	231-234	53.65; 5.16; 4.57 / 53.58; 5.11; 4.60	266, 358
XIX	$C_{40}H_{45}N_3O_{19}S$	903.86	87	229-233	53.15; 5.01; 4.64 / 53.09; 4.96; 4.59	266, 359
XX	$C_{39}H_{43}N_3O_{19}S$	889.83	88.51	200-204	52.64; 4.87; 4.72 / 52.68; 4.90; 4.73	266, 359
XXI	$C_{38}H_{44}N_2O_{20}S$	880.82	85.06	279-282	51.81; 5.03; 3.18 / 51.78; 5.07; 3.20	263, 357
XXII	$C_{38}H_{44}N_2O_{19}S$	864.82	83.67	277-280	52.77; 5.12; 3.23 / 52.82; 5.09; 3.25	263, 356
XXIII	$C_{37}H_{42}N_2O_{19}S$	850.80	87.05	285-287	52.23; 4.97; 3.29 / 52.18; 4.92; 3.32	263, 356
XXIV	$C_{36}H_{40}N_2O_{19}S$	836.77	84.29	281-285	51.67; 4.81; 3.34 / 51.71; 4.80; 3.36	263, 358
XXV	$C_{40}H_{44}N_4O_{19}S$	916.86	93.72	290-293	52.40; 4.83; 6.11 / 52.36; 4.79; 6.08	260, 356
XXVI	$C_{40}H_{44}N_4O_{18}S$	900.86	92.86	288-292	53.33; 4.92; 6.21 / 53.38; 4.96; 6.23	259, 357
XXVII	$C_{39}H_{42}N_4O_{18}S$	886.83	91.06	292-295	52.82; 4.77; 6.31 / 52.88; 4.81; 6.39	260, 357
XXVIII	$C_{38}H_{40}N_4O_{18}S$	872.81	90.86	283-287	52.29; 4.61; 6.41 / 52.34; 4.56; 6.37	261, 357
XXIX	$C_{41}H_{46}N_4O_{18}S$	930.89	89.76	244-247	52.90; 4.98; 6.01 / 53.00; 5.03; 6.03	264, 357
XXX	$C_{41}H_{46}N_4O_{18}S$	914.89	88.35	252-256	53.82; 5.06; 6.12 / 53.78; 5.02; 6.13	262, 357
XXXI	$C_{40}H_{44}N_4O_{18}S$	900.86	88.98	248-253	53.33; 4.92; 6.21 / 53.39; 4.99; 6.24	261, 357
XXXII	$C_{39}H_{42}N_4O_{18}S$	886.83	87.45	211-216	52.82; 4.77; 6.31 / 52.77; 4.72; 6.33	261, 357
XXXIII	$C_{39}H_{43}N_3O_{19}S_2$	921.89	84.87	289-293	50.81; 4.70; 4.55 / 50.73; 4.60; 4.59	267, 359
XXXIV	$C_{39}H_{43}N_3O_{18}S_2$	905.89	86.21	270-274	51.70; 4.78; 4.63 / 51.61; 4.70; 4.58	267, 358
XXXV	$C_{38}H_{41}N_3O_{18}S_2$	891.87	85	297-300	51.17; 4.63; 4.71 / 51.01; 4.56; 4.62	267, 358
XXXVI	$C_{37}H_{39}N_3O_{18}S_2$	877.84	83.98	294-297	50.62; 4.47; 4.78 / 50.69; 4.55; 4.83	267, 359

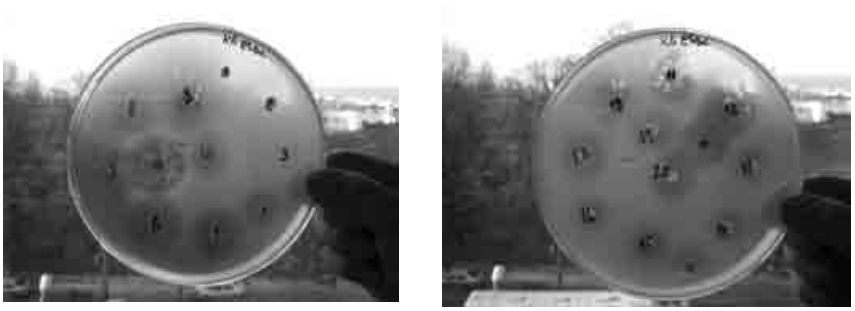


Figure 2

Rutin derivatives activity against ESBL-producing *Klebsiella pneumoniae pneumoniae 1*

Table II

Antibacterial activity of rutin derivatives I-XXXVI

Compound No.	*S. aureus* ATCC 25923	*S. aureus* methycillin-resistant	*S. lutea* ATCC 9341	*B. cereus* ATCC 14579	*B. subtilis*	*E. coli* ATCC 25922	Pseudomonas *aeruginosa*	*ESBL-producing Klebsiella pneumoniae pneumoniae* 1
I	12	16	29	20	20	14	12	0
II	18	0	30	22	20	17	0	7
III	7	10	22	22	26	14	10	0
IV	0	16	25	20	26	14	0	12
V	0	10	25	20	15	15	0	21
VI	10	10	30	25	25	0	0	21
VII	0	14	30	25	20	0	0	21
VIII	11	10	30	26	22	16	0	16
IX	21	0	32	20	20	17	0	15
X	21	0	30	18	0	0	0	13
XI	21	0	28	20	20	0	0	24
XII	16	0	30	25	20	10	0	26
XIII	12	0	25	10	18	7	0	10
XIV	15	10	22	0	18	9	0	0
XV	17	10	21	10	15	14	0	0
XVI	11	0	17	0	15	9	0	0
XVII	0	0	27	19	20	10	0	0
XVIII	7	0	25	20	0	0	0	10
XIX	7	0	25	19	16	0	0	15
XX	11	0	25	20	20	0	0	15
XXI	0	0	10	0	0	0	0	15
XXII	0	0	10	0	15	10	0	12
XXIII	21	10	22	0	15	12	0	11
XXIV	0	0	25	0	17	10	0	12
XXV	0	0	30	20	15	20	0	10
XXVI	0	0	30	20	20	21	0	16
XXVII	0	0	29	17	22	20	0	19
XXVIII	0	0	30	25	21	17	0	19
XXIX	0	0	30	19	20	19	0	12
XXX	0	0	32	22	19	20	0	14
XXXI	0	0	32	23	23	20	0	17
XXXII	0	0	31	21	22	20	0	17
XXXIII	13	10	30	20	20	19	0	15
XXXIV	0	0	31	20	20	19	0	15
XXXV	0	0	31	20	16	17	0	19
XXXVI	17	11	31	26	27	17	0	15
Tetracycline 30 µg	26	16	30	20	25	17	0	30
Co-trimoxazole 25 µg	17	20	30	19	20	21	0	21
Sulfanilamide 200 µg	15	0	25	20	15	15	0	14

The data obtained in the quantitative antimicrobial activity are presented in Table III. The results show again that the XI, XII, XIII and XV derivatives have good antibacterial activity and could be used as antimicrobial agents.

In vivo antibacterial activity evaluation showed protective effects against 507 strain *Klebsiella pneumoniae* and 558 strain of *Streptococcus pneumoniae* infection. The most active compounds are the rutin-sulfapyridine XV and XIII derivatives (figure 3).

Table III

MIC and MBC values for XI, XII, XIII and XV rutin derivatives (mg/L)

Compound	tested strain			
	S. aureus ATCC 25923		*E. coli* ATCC 25922	
	MIC	MBC	MIC	MBC
XI	32	64	*nt	*nt
XII	8	32	*nt	*nt
XIII	32	64	*nt	*nt
XV	8	32	16	64
Sulfapyridine	16	32	nt*	nt*
Sulfachloropyridazine	16	32	nt*	nt*

**nt = not-tested*

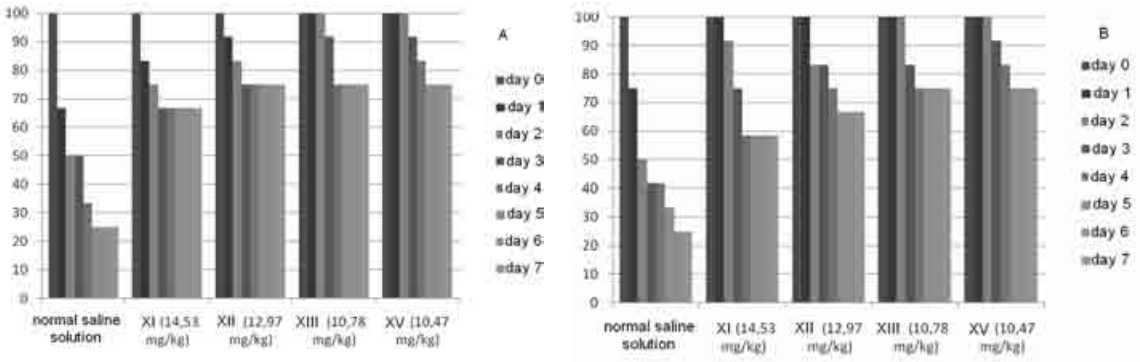


Figure 3

Survival rate after experimental infection with 507 *Klebsiella pneumoniae* (A) and 558 *Streptococcus pneumoniae* (B)

Conclusions

Thirtysix new water-soluble rutin-sulphonamide derivatives were synthesised. All the compounds were characterized by IR and UV spectral methods and by elemental analysis. The *in vitro* and *in vivo* antibacterial assays showed a good antibacterial activity. The most active compounds were the XI, XII, XIII and XV derivatives, which have *in vitro* antibacterial activity comparable with cotrimoxazole activity. The XV and XIII

derivatives also shown good protective effects against experimental infection on mice.

Acknowledgements

The authors are grateful to the board of the „Gr. T. Popa" University of Medicine and Pharmacy, Iasi, for financial support of this research (internal project no. 16408 / 2009).

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Manuscript received: October 6th 2010