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Original Research Article

Ames test and SOS Chromotest to Evaluate the Genotoxicity Effect of Synthesized Series of Antibacterial Sulfonamides

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Abstract

Genotoxic property of four new antibacterial sulfonamides **1a-d** has been evaluated in this study using two standard genotoxicity assays: the *Salmonella typhimurium* mutagenicity assay or Ames test based on the use of *Salmonella* strains TA100, TA98 and TA1535, treated with and without metabolic activation (S9 mix fraction) and the SOS *Chromtest*^{TM} Kit assay using *Escherichia coli* PQ 37.

From the results of the Ames test we note that only **1c** (N-(phenyl) sulfamide) showed no genotoxic effect, contrary to **1a** [(N-(4-methoxyphenyl) sulfamide], **1b** [(N-(3-fluorophenyl) sulfamide] and **1d** [(N-(phenylethyl) sulfamide] that have showed genotoxic effect with and without metabolic activation. Results of the SOS Chromotest confirmed these. Sulfonamides **1a, 1b** and **1d** expressed the genotoxic potential by stimulating the production of β-galactosidase. The genotoxic effect of these molecule is strictly linked to their carcinogenic potential. So, from our results, we suggested that only compound 1c was non-genotoxic and safe to be tested, eventually, *in vivo*. Furthermore, we conclude that genotoxic effect depends essentially on the structure and composition of the molecule.

Keywords:
Sulfonamides, Genotoxicity, Ames test, SOS Chromotest.

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1. Introduction

It is widely recognized that there is an increase in antibiotic-resistant pathogenic microorganisms becoming one of the most vital threats to the healthcare sector. Multidrug-resistant bacteria (MDR) that are deadly pathogenic are rising day by day and pose one of the biggest challenges and very serious threat of health mankind [1], [2]. In order to fix that, we have an urgent need of new antibacterials agents, with an innovative mechanism of action [3]. Nowadays many biotechnology companies as well as many scientists and researchers are involved in the search for new drugs effective and safe for human health [4]. Drugs are a poignant example in the study of toxicology. Although therapeutic and very beneficial at certain doses, they are not without deleterious side effects and can kill at higher doses. To validate these new compounds, the researchers used several assessment tests recommended by several health organizations [5], namely, tests for *in vitro* and *in vivo* biological activities.

Sulfonamides are among the most widely used antibacterial agents in the world. They were the first effective chemotherapeutic agents used systematically for the prevention and cure of bacterial infections in humans and some animals, mainly because of their low cost, low toxicity and excellent activity against bacterial diseases [6]; they were a promising drug candidate for treatment of bacterial infections that's why it is important to characterize their genotoxicity effect.

In this study, we used two standards genotoxicity assays (Ames test or *Salmonella typhimurium* mutagenicity assay and the SOS *Chromotest*^{TM}) to assessed preclinical safety of a new series of four antibacterial sulfonamides [7].

The genotoxicity tests are carried to avoid the DNA damage that is considered as the initiating by which a molecule causes hereditary effects (point mutation or chromosomal damage) and cancer [8], [9]. Thus, the evaluation of the genotoxic potential of newly synthesized molecules,such as sulfonamides, constitutes one of the very important preliminary steps in the course of thesafety assessment and regulatory control of chemicals [10].

The Ames test, also called Salmonella/microsome assay played a critical role in the spread of bacterial tests [11], [12]. It measures reverse mutations from histidine auxotrophy to prototrophy in several especially constructed mutants. It is now the most extensively used as well as the bacterial short-term test validated on the largest scale in a number of laboratories [13]. The SOS chromotest is a colorimetric assay which measures the expression of genes induced by genotoxic agents, in *Escherichia coli*, by means of a fusion with the structural gene for the β-galactosidase. The main advantages of the SOS chromotest are practical. A single strain is required. A quantitative colorimetric response is obtained within a few hours [14], [15].

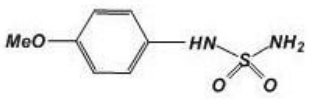
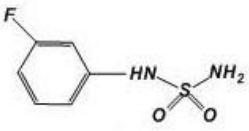
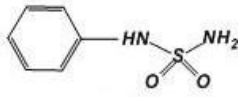
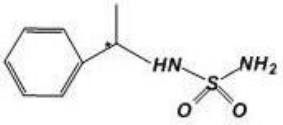
2. Material and methods

2.1. Tested sulfonamides

Tested sulfonamide compounds **1a-d** were synthesized by the Laboratory of Applied Organic Chemistry, Badji Mokhtar University, Annaba-Algeria (Table 1). A Serial dilutions of sulfonamides **1a-d** were prepared in acetone ranged from 0.5 to 512 µg/ml.

Two commercial drugs were used as positive controls and were diluted in the same manner: Control 1: Bactrim, sulfamethoxazole-trimethoprime (400/80mg) (Laboratoire Roche, France), and control 2: Sulfaguanidine (500 mg) (Merck, France).

Table 1: Chemical structure of the four tested antibacterial sulfonamides **1a-d**.

	
1a : N-(4-methoxyphenyl) sulfamide **(**$C_7H_{10}N_2O_3S$ **)**	**1b : N-(3-fluorophenyl) sulfamide** **(**$C_6H_7N_2O_2S\ F$**)**
	
1c : N-(phenyl) sulfamide **(**$C_6H_8N_2O_2S$ **)**	**1d : N-(phenylethyl) sulfamide** **(**$C_8H_{12}N_2O_2S$ **)**

2.2. Genotoxicity assessment

Ames Test: Phenotypes and genotypes of tester strains

The *Salmonella typhimurium (S. typhimurium)* tester strains **TA98, TA100** and **TA1535** used for the assay were obtained from MOLTOX, Molecular Toxicology Inc, USA. Their phenotypeswas listed in Table 2; the genotypes and the type of mutation detected are listed in Table 3.

Table 2: *S. typhimurium* tester strains' phenotype [10].

Strains	Allele	Reversion event	DNA target
TA98	His D3052	Frameshifts	–C–G–C–G–C–G–C–G–
TA100	cHis G46	Base-pair substitution	–G–G–G–
TA1535			

Table 3: *S. typhimurium* tester strains' genotypes and the type of mutation [10].

Diagnostic	TA98	TA100	TA1535	Medium
his	+	+	+	-L-his
rfa	+	+	+	+L-his(CV disc)
R-factor	+	+	+	+Amp
pAQ1	_	_	_	+Amp, +Tet
urvB	+	+	_	+ Nutrient agar
Revertants	15–75	60–220	5–20a	
Plasmid	pKM101	pKM101	No plasmid	

Notes: + growth; _no growth.

a : No metabolic activation.

2.2.1. Metabolic activation

S9 microsomal fraction was obtained from MOLTOX, Molecular Toxicology Inc, USA, and used as a metabolic activation system.

2.2.2. Experimental method

The preincubation assay was performing as described by Mortelmans and Zeiger, [16]. It is a modification of the standard plate incorporation assay [11], [12]. Prior to plating on GM agar medium, it entails briefly exposing the tester strains in a tiny volume containing the test agent with and without S9 mix.

In sterile tubes, we added in the following order with stirring after each addition: 0.05 ml of the tested sulfonamide; 0.10 ml of the overnight culture of the *Salmonella* strain to a density of about $1 - 2x10^9$ CFU/ml. The test was performed with and without metabolic activation by adding 0.50 ml of the S9 mix.

The mixture was incubated at 37°C for 20 min with gentle agitation. 2ml of molten top agar maintained at 40 to 43°C was added to each tube.

Test tube contents were combined and then poured onto the surface of GM agar plates. The plates were incubated at 37°C for 48h after the top agar has hardened.

The positive controls were included as 4-NQO (2,5 µg/ml/plate) for TA98 and the sodium azide (5 µg/ml/plate) for TA100 and TA1535. Commercial drugs (Bactrim, sulfamethoxazole-trimethoprime and Sulfaguanidine) were also screened for their genotoxicity in the same manner.

All tests were performed in duplicate, and experiment was repeated three times. The results are then expressed as the number of revertant colonies per plate after the colonies have been counted.

2.2.3. Data analysis

The average number of revertant colonies per plate and the standard deviation for the tested sulfonamides, positive and negative controls were calculated. Compared with the negative con-

trols, positive results can be judged if the number of revertant colonies of the compounds plate in any strain with or without metabolic activation has a dose-related increase or the number of revertant colonies per plate was twofold in the number of His revertants/plate. Otherwise, negative result was defined.

2.2.4. The SOS Chromtest™

The SOS *Chromtest™* is a convenient approach for the detection of genotoxic activity and genotoxic materials in environmental water, sediment, air, chemicals, food components, cosmetics and biological fluids. Genotoxic materials may be hazardous due to their ability to induce mutations and cancerous transformation of normal cells [15].

The SOS chromotest was performed in microplates according to the EBPI SOS-*CHROMOTEST™* kit manual.

According to the manufacture instructions, the different reagents, the freshly prepared bacterial suspension and the positive control were added to the different wells of the microplate.

The micro-plate was incubated at 37°C for 2 hours. During this time, the bacteria are exposed to the material which contains the suspected genotoxins. After incubation a chromogenic substrate will be added.

In this test we have used the simultaneous activity check of β-galactosidase and alkaline phosphatase. The blue chromogen was transferred to the dry alkaline phosphatase substrate and then 100µl was added into each well of the plate. The plate was incubated again at 37°C for 60 to 90 min until a green color appears. Absorbance (OD) was read at 615nm to measure genotoxic activity and at 405nm to determine viability of bacteria.

2.2.5. Calculating the SOSIP

The SOS inducing potency (SOSIP) was given in the following equation:

$$SOSIP = 10X(OD_a - O_{Db})/(C_a - C_b) \quad (1)$$

The expression $(C_a - C_b)$ in equation (1) is entered in nano-moles per reaction well.Equation (2) transforms microgram concentration values to the required nanomole unitage:

$$C = CONCXVOL/MW \quad (2)$$

CONC: Concentration of tested material in µg/ml, VOL: Volume of the tested material solution in the well expressed in microlitres MW: Molecular weight of the tested material.

If the SOSIP is equal to 0 or smaller, it may mean that the material is not genotoxic.

3. Results

3.1. Ames test (en rouge faute c rectifiée)

Results of the genotoxicity effect of the new series of sulfonamides **1a-d** tested using *S. typhimurium* strains, with and without metabolic activation, were shown in the 4, 5, 6 and 7.

Sulfonamide **1a** [(N-(4-methoxyphenyl) sulfamide], showed genotoxic effect against strain TA100 with and without metabolic activation (S9). With all concentrations, high number of revertant colonies was seen. Without metabolic activation, the values vary between 922 ± 5,5 (at concentration 0,5µg/ml) and 7232 ± 10,14 (at concentration 512µg/ml). With metabolic activation, the number of revertant colonies is more important, varying between 1910,33 ± 15 (at concentration 0,5µg/ml) and 8871 ± 10,53 (at concentration 512µg/ml) (Table 4), (Figure 1).

This compound expressed a genotoxic effect by inducing a base-pair substitution mutation. No genotoxic effect was observed with TA98 and TA1535 strains.

Regarding sulfonamide 1b [(N-(3-fluorophenyl) sulfamide], genotoxic effect was expressed with TA1535 strain, with and without metabolic activation. The number of revertant colonies vary between 617 ± 10 (at concentration 0.5 g/ml) and 1392.66 ± 11.52 (at concentration 512 µg/ml) without metabolic activation.

With metabolic activation the number vary between 1457 ± 20 (at concentration 0.5 µg/ml) and 3211.33 ± 17.52 (at concentration 512 µg/ml) (Table 5).

Sulfonamide 1b induced pair-base substitution mutation also. No genotoxic effect was noted with TA100 and TA98 strains.

No genotoxic effect was observed for the compound 1c (N-(phenyl) sulfamide) with the three tested strains of *S. typhimurium* TA100, TA98 and TA1535 with and without metabolic activation (Table 6).

For the sulfonamide 1d [(N-(phenylethyl) sulfamide], genotoxic effect was expressed with TA100 and TA1535, with and without metabolic activation. Without metabolic activation, the number of revertant colonies of TA100 strain was about 586 ± 5 (at concentration 0.5 µg/ml) and 1873.66 ± 21.52 (at concentration 512 µg/ml).

With the metabolic activation, it vary between 936.66 ± 10.96 (at concentration 0.5 µg/ml) and 4236.33 ± 11.15 (at concentration 512 µg/ml). For TA1535 strain, Number of revertant colonies was about 1127 ± 12.52 and 5781.66 ± 23.15 for 0.5 et 512 µg/ml concentrations respectively without metabolic activation. With this latter, the number vary between 2342.33 ± 10,52 and 8964 ± 22 colonies (Table 7).

All genotoxic molecules expressed their genotoxic effect on TA100 or TA1535 strains or both, reflecting base-pair substitution mutation. Bactrim (control 1) and Sulfaguanidine (control 2) showed no genotoxic effect with the three *S. typhimurium* strains, with and without metabolic activation, showing a low number of revertant colonies (Tables 8 and 9).

Table 4: Number of revertant colonies of *S. typhimurium* **TA100, TA98** and **TA1535** strains towards sulfonamide **1a** with and without metabolic activation (S9 mix).

Concentrations (µg/ml)	Bacterial strains					
	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
512	7232 ± 10.14	8871 ± 10.53	18.66 ± 1.15	30.66 ± 0.57	58.33 ± 1.52	60.66 ± 1.15
256	6939.66 ± 13.57	8646 ± 11.59	21 ± 1	27 ± 1	58.33 ± 0.57	56 ± 1
128	5997 ± 2.64	7653 ± 10.53	21 ± 1	22.33 ± 0.57	56.66 ± 1.15	56.33 ± 2.08
64	5872.33 ± 11.59	7498.33 ± 9.86	21.66 ± 1.52	20 ± 0	55.33 ± 1.15	50 ± 1
32	5673 ± 10.53	7133 ± 12.12	20 ± 0	20.66 ± 0.57	53 ± 1	51 ± 1
16	4190.33 ± 5.5	6231 ± 11.53	20 ± 1	19 ± 1	51 ± 1	47.66 ± 0.57
8	2664 ± 8.71	5479.66 ± 10.52	19 ± 1	17 ± 1	50 ± 0	45.66 ± 0.57
4	2432.33 ± 9.60	4671.66 ± 11.59	18.33 ± 1.15	17 ± 1	50 ± 1	43 ± 1
2	945.66 ± 9.01	4215 ± 5.5	14 ± 1	16.66 ± 1.15	43.33 ± 1.15	41 ± 1
1	920.33± 5.5	2453 ± 10	14 ± 1	15.66 ± 1.15	39.66 ± 0.57	39 ± 1
0.5	922 ± 5.5	1910.33 ± 15	10 ± 0	14.33 ± 0.57	35 ± 1	36 ± 1
4-NQO (2.5 µg/ml)	/	/	**1700 ± 20**	/	/	/
Sodium azide (5µg/ml)	**1200 ± 20**	/	/	/	**1200 ± 20**	/

(-S9): Without metabolic activation.

(+S9): With metabolic activation.

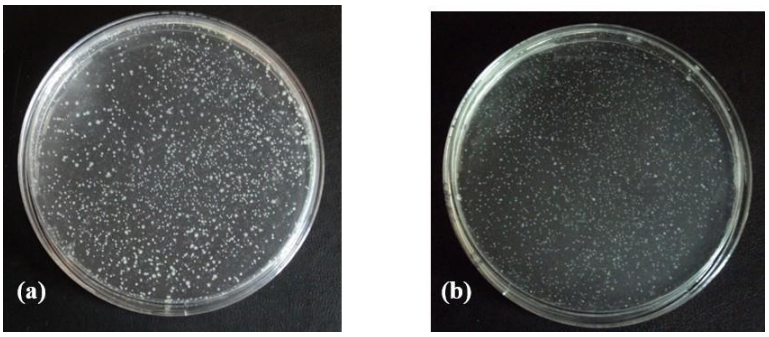


Figure 1: Number of revertant colonies obtained with TA100 strain at concentration 512 µg/ml against sulfonamide 1a, without (a) and with (b) metabolic activation.

Table 5: Number of revertant colonies of *S. typhimurium* **TA100, TA98** and **TA1535** strains towards sulfonamide **1a** with and without metabolic activation (S9 mix).

Concentrations (µg/ml)	Bacterial strains					
	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
512	96.33 ± 2.57	66 ± 0.57	50.66 ± 0.57	66 ± 1	1392.66 ± 1.52	3211.33 ± 17.52
256	85.66 ± 1.15	58.33 ± 1.15	46.66 ± 1.52	60.66 ± 1.15	1281 ± 10	3127.66 ± 20.52
128	74.66 ± 1.15	56 ± 1	47.33 ± 1.15	56.66 ± 1.52	1017 ± 13	2875.66 ± 20.51
64	37.33 ± 0.57	48 ± 1	38.66 ± 1.15	50.33 ± 0.57	1016.66 ± 5.52	2579.33 ± 21.52
32	39.33 ± 1.15	42.33 ± 1.52	35.,66 ± 0	50.66 ± 1.15	1001.66 ± 10.52	2431.33 ± 18.52
16	19 ± 1	21 ± 1	36 ± 0.57	43.66 ± 1	959.66 ± 8.57	2400 ± 21
8	19.66 ± 0.57	19.66 ± 0.57	35.6 6± 1.15	41 ± 0.57	805 ± 10	2217.66 ± 10.57
4	17 ± 1	17 ± 1	35 ± 1	35 ± 0	741.33 ± 8.57	2110 ± 14.5
2	10.33 ± 0.57	13.66 ± 0.57	34.66 ± 0.57	33 ± 1	706 ± 10.5	1712 ± 22.1
1	6.66 ± 0.57	10.33 ± 0.57	31 ± 1	29.33 ± 1.15	686.66 ± 12.57	1561.66 ± 10.57
0.5	4 ± 0	8 ± 0	22.66 ± 0.57	24.33 ± 0.57	617 ± 10	1457 ± 20

Table 6: Number of revertant colonies of *S. typhimurium* **TA100**, **TA98** and **TA1535** strains towards sulfonamide **1c** with and without metabolic activation (S9 mix).

Concentrations (µg/ml)	Bacterial strains					
	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
512	69 ± 1	82 ± 1	38.33 ± 0.57	37.33 ± 1.15	56.33 ± 1.15	56.33 ± 0.57
256	37 ± 1	66.66 ± 0.57	36 ± 1	34.33 ± 0.57	47.33 ± 0.57	55 ± 2
128	35 ± 1	54.66 ± 0.57	36 ± 1	34 ± 1	43.66 ± 1.52	53 ± 0
64	30.33 ± 0.57	49 ± 1	35,33 ± 1.15	33 ± 1	43 ± 1	47 ± 1
32	21.66 ± 1.52	44.66 ± 0.57	32.66 ± 1.15	31.66 ± 0.57	42.66 ± 1.15	44 ± 1
16	19 ± 1	37.66 ± 0.57	30.66 ± 1.15	31.33 ± 1.15	41.66 ± 1.52	42.66 ± 1.15
8 4	16 ± 0	31 ± 1	30.33 ± 0.57	30.66 ± 1.15	35.33 ± 1.15	41.33 ± 1.15
2	14.66 ± 0.57	28.66 ± 1.52	30 ± 0	29.66 ± 0.57	31.33 ± 1.15	34.33 ± 0.57
1	13.33 ± 1.15	25 ± 0	30 ± 1	29.33 ± 1.15	29.33 ± 1.15	32.66 ± 1.15
0.5	11.33 ± 1.15	20 ± 1	26 ± 1	26.66 ± 1.15	28.33 ± 1.52	30 ± 0
	11.66 ± 1.52	17.66 ± 0.57	25 ± 1	24.66 ± 0.57	20 ± 0	23 ± 1

Table 7: Number of revertant colonies of *S. typhimurium* **TA100**, **TA98** and **TA1535** strains towards sulfonamide **1c** with and without metabolic activation (S9 mix).

Concentrations (µg/ml)	Bacterial strains					
	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
512	1873.66 ± 21.52	4236.33 ± 11.15	104.33 ± 1.15	111 ± 1	5781.66 ± 23.15	8964 ± 22
256	1429.66 ± 11.52	3987 ± 18	82.33 ± 0.57	87 ± 1	4962 ± 17.73	8821 ± 19.73
128	1297 ± 17	3871 ± 18.73	60.33 ± 0.57	71 ± 1.73	4741.33 ± 20.3	8677.33 ± 20.08
64	1238.33 ± 18.52	3655.66 ± 10.57	45 ± 1	51.66 ± 0.57	4337.66 ± 14.52	8418.33 ± 21.52
32	1221 ± 21.73	3438 ± 16	41.66 ± 1.52	46 ± 1	3652.33 ± 12.51	7854 ± 11.73
16	1187.33 ± 15.15	2949.66 ± 17.52	26 ± 0	31 ± 1	3432 ± 20	7386 ± 19
8	1125 ± 12	2802 ± 20	22.33 ± 1.15	24.66 ± 0.57	2372.66 ± 20.51	5763 ± 14.73
4	731.66 ± 10.52	1876.66 ± 11.52	19.66 ± 0.57	23 ± 1	1796 ± 13	4019 ± 12
2	689 ± 8.54	1430.66 ± 13.78	16.66 ± 0.57	21 ± 1	1537.66 ± 10.52	3882 ± 12
1	586.66 ± 7.63	1106 ± 5.29	11 ± 0	14.33± 0.57	1322.66 ± 12.51	3190 ± 20
0.5	586 ± 5	936.66 ± 10.96	6.66 ± 0.57	11 ± 0	1127 ± 12.52	2342.33 ± 10.52

3.2. The SOS Chromtest^{TM}

The obtained results of the SOS Chromotest were in agreement with those obtained with the Ames test. Dose-dependent genotoxicity effect was showed, with sulfonamides **1a**, **1b** and **1d**,

Table 8: Number of revertant colonies of *S. typhimurium* **TA100**, **TA98** and **TA1535** strains towards sulfonamide **1c** with and without metabolic activation (S9 mix).

Concentrations (µg/ml)	Bacterial strains					
	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
512	165.33 ± 1.15	180.66 ± 1.15	80.66 ± 1.15	99 ± 1	49.33 ± 0.57	60 ± 0
256	141 ± 1	170.33 ± 1.15	76.66 ± 1.15	91.33 ± 1.15	40.66 ± 1.15	55.66 ± 0.57
128	127.66 ± 0.57	145.33 ± 1.15	71.33 ± 1.15	84.66 ± 0.57	36 ± 1	50.66 ± 1.15
64	98.66 ± 1.52	127.66 ± 1.52	65 ± 1	79.66 ± 0.57	33 ± 0	49 ± 1
32	88 ± 0	121 ± 0	57 ± 1	72 ± 0	30.33 ± 0.57	42.33 ± 1.15
16	76.33 ± 0.57	116 ± 1	50.66 ± 0.57	67.33 ± 0.57	27.33 ± 0.57	38 ± 0
8	59.33 ± 0.57	99 ± 1	43 ± 0	60.66 ± 0.57	23.66 ± 0.57	34 ± 1
4 2	53 ± 3	90.33 ± 0.57	39.66 ± 0.57	55 ± 0	20.33 ± 0.57	29.66 ± 0.57
1	49.66 ± 1.52	87 ± 1	34.33 ± 0.57	51 ± 4	16 ± 0	28 ± 0
0.5	45.33 ± 0.57	80.33 ± 0.57	30 ± 1	46.33 ± 1.52	15 ± 0	25 ± 1
	37.33 ± 1.15	74 ± 1	24 ± 0	40 ± 2	12.66 ± 1.15	22 ± 1

Table 9: Number of revertant colonies of *S. typhimurium* **TA100**, **TA98** and **TA1535** strains towards sulfonamide **1c** with and without metabolic activation (S9 mix).

Concentrations (µg/ml)	Bacterial strains					
	TA 100		TA 98		TA 1535	
	(-S9)	(+S9)	(-S9)	(+S9)	(-S9)	(+S9)
512	63.33 ± 1.15	89 ± 1	46 ± 0	66 ± 1.73	54 ± 1	67 ± 1
256	56.33 ± 0.57	84 ± 1	41.66 ± 0.57	60.66 ± 1.15	50.66 ± 0.57	62.66 ± 0.57
128	55 ± 2	80.66 ± 1.15	39 ± 1	55 ± 0	46.66 ± 0.57	60 ± 5
64	52.33 ± 0.57	78 ± 1	35.66 ± 0.57	51 ± 1	41 ± 0	56.66 ± 1.15
32	50 ± 3	73.66 ± 1.52	32.33 ± 0.57	48.33 ± 0.57	35 ± 1	51.66 ± 1.15
16	46.66 ± 0.57	69 ± 1	31.33 ± 0.57	43.33 ± 1.15	30 ± 0	47.33 ± 0.57
8	40.66 ± 1.15	67 ± 1	30 ± 0	39.33 ± 1.15	28.66 ± 0.57	43.33 ± 1.15
4	34.66 ± 0.57	63 ± 1	28.66 ± 0.57	38.66 ± 1.15	26.33 ± 0.57	40 ± 2
2	30.33 ± 0.57	55 ± 1	25.33 ± 0.57	35.66 ± 0.57	25.66 ± 0.57	37.33 ± 0.57
1	28 ± 0	50 ± 0	20.33 ± 0.57	33 ± 0	25 ± 0	31.66 ± 1.52
0.5	25.66 ± 0.57	43 ± 2	16 ± 0	28.33 ± 1.52	21.33 ± 0.57	29 ± 1

by the appearance of green color in the microplate wells due to the expression of β-galactosidase which is linked to the induction of the SOS system. The exponential values of the OD 605 were comparable to those of the positive control 4-NQO which also expresses a green color due to the expression of β-galactosidase.

Sulfonamide **1c** was not genotoxic. The results with the different concentrations are comparable to those of the negative control, revealing a yellow color of the microplate wells at OD 405. The yellow color reveals the viability of *Escherichia coli* PQ37 strain and is due to the expression of alkaline phosphatase measured at OD 405.

The SOSIP is calculated for sulfonamide compounds **1a**, **1b** and **1d** which gave a positive colorimetric genotoxic response (green color) as well as dose-dependent effect at OD 605. For the sulfonamides **1a**, **1b** and **1d**, the SOSIP were 8; 5,76 and 8 respectively (Table 10). A SOSIP less than or equal to 0 means that the compound is not genotoxic.

Regarding the two controls used in our work, no genotoxic effect was reported with the SOS chromotest.

Table 10: SOSIP results for sulfonamides **1a-d** and the two controls (Bactrim and Sulfaguanidine).

Sulfonamides	SOSIP	Genotoxicity effect
4-NQO	28	(+)
1a	8	(+)
1b	5,76	(+)
1c	0	(-)
1d	8	(+)
Control 1	0	(-)
control 2	0	(-)

4. Discussion

Information on genotoxicity is a key component in risk assessment of chemicals in general, including those used in food and feed, consumer products, human and veterinary medicines, and industry [17]. Genotoxicity tests are conducted to highlight possible damage to DNA that can be considered as the initiator whereby a molecule can cause hereditary effects (point mutations or chromosomal alterations) and cancer [8].Thus, the assessment of genotoxic potential of newly synthesized molecules is one of the very important preliminary steps in the framework of the evaluation of the preclinical safety and regulatory control chemicals. The genotoxicity testing is done mainly by *in vitro* assays [10]. Bacteria played an essential role in the origin of short-term tests designed to detect genotoxic agents, because they grow rapidly in simple defined media. In fact, bacterial short-term tests are among the simplest, quickest and less expensive to conduct [18], [19].

To evaluate the genotoxicity effect of this new synthesized series of four sulfonamides **1a-d**, we used two standard assays: Ames test and SOS Chromotest.

The capacity of the Ames test to identify carcinogens is higher than that of the SOS Chromotest. However, because of the lower number of false positive compounds in the SOS Chromotest, both can complement each other [20].

Our results showed that sulfonamides **1a** [(N-(4-methoxyphenyl) sulfamide], **1b** [(N-(3-fluorophenyl) sulfamide] and **1d** [(N-(phenylethyl) sulfamide] were genotoxic compounds, with the Ames test (with and without metabolic activation) confirmed by the SOS Chromotest. On the other hand, only sulfonamide **1c** (N-(phenyl) sulfamide) showed no genotoxic effects with both tests.

We noted that the three genotoxic compounds are composed by the phenol group plus other compounds, unlike molecule **1c** composed only by phenol. Therefore, we can conclude that this combination is at the origin of this genotoxic effect.

Indeed, several studies have been conducted on chemicals that may pose a genotoxic effect . Quillardet and Hofnung (1993), mentioned substances that have been evaluated for their genotoxic potential with the Ames test and the SOS Chromotest. Among the results, com-

pounds containing benzene, fluor and oxide demonstrated genotoxicity, as well as compounds containing phenyl, ethyl, methyl and fluorine in combination with other compounds [21], which corresponded to our results.

Broschinski et al., (1998) conducted a comparative analysis of a total of 776 new substances marketed and notified by the German government between 1982 and 1997, to characterize their genotoxic effect. Results showed that 36 substances have genotoxic effect correlating with their chemical properties, such as the presence of methoxy or ethoxy substituents [22].

5. Conclusion

Sulfonamides are an important class of antibiotic drugs with a wide range of activity, being very effective against Gram positive and Gram-negative bacteria.

These synthetic compounds were known for their structural variability. Indeed, each year new sulfonamides are synthesized and tested for their biological activities, starting with the evaluation of tocixicity (cytotoxicity and genotoxicity). Our new series of sulfonamides, showed, in a later work [23], a good antibacterial activity were evaluated for their genotoxicity. Hence, we concluded that only compound **1c** presented a good drug candidate and can be tested, *in vivo*, to determine its pharmacodynamics and pharmacokinetics, and can be used as an antibiotic.

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