# Synthesis and Theoretical Calculations of 5-Aminosalicylic Acid Derivatives as Potential Analgesic Agents

Correa-Basurto José[a,b], M.C. Rosales-Hernández[a], Ramírez-San Juan Eduardo[c], Badillo Abigail[a], Z. Gómez-Castro Carlos[a], L.H. Fabila-Castillo[d] and Trujillo-Ferrara José[a,*]

[a]*Sección de Estudios de Posgrado e Investigación, Departamento de Bioquímica y* [b]*Departamento de Farmacología, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón,11340 México City, México;* [c]*Departamento de Fisiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México city, 11340, México;* [d]*Departamento de Inmunologia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México city, 11340, México*

**Abstract:** 5-Aminosalicylic acid is one of the drugs most commonly used for inflammatory bowel disease treatment, although its use is limited due to side effects. The aim of this work was to synthesize four 5-ASA derivatives (**1**-**4**) and analyze their pharmacological effects. The compound structures were elucidated by spectral (IR and $^{1}H$ and $^{13}C$-NMR) analysis, and their analgesic effects and lethal doses 50 ($LD_{50}$) were evaluated in the mouse model. In addition, their Log Ps and affinities for both cyclooxygenase enzymes (COX I and COX II) were evaluated through theoretical calculations.

All compounds showed analgesic activities from 0.1 mg/Kg to 16 mg/Kg in the mouse model. The imides showed more affinity by COX enzymes and their Log Ps were the highest. The docking calculations showed that all compounds have good affinities for COX I and COX II (≅ 1 μM), making π–π, van der Waals interactions and hydrogen bonds. The toxicities of all compounds were low, judging by the $LD_{50}$.

Finally, the docking analysis show that the compounds act on COX enzymes and their analgesic effects could be mediated in part by the inhibition of these enzymes.

**Key Words:** 5-ASA, COX-inhibitor, docking, amides, imides.

## INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a serious disorder of the lower gastrointestinal tract in which tissue damage and inflammation lead to bowel impairment. Both are chronic, progressive diseases associated with considerable pain, abdominal cramping, persistent diarrhea, vomiting, gastrointestinal bleeding, anemia, fever, weight loss and secondary infections [1]. 5-Aminosalicylic acid (5-ASA) is one of the principal drugs administered for the IBD treatment which is used as a pro-drug [2-6]. However, its use has been limited due to adverse effects [7-10]. The mode of action of 5-ASA is unclear, despite the well-known ability of 5-ASA to scavenge radicals and inhibit the production of leukotrienes and prostaglandins [11-13]. One theory holds that this mechanism is related to the cyclooxygenase inhibitor effect [13], while another sustains that it could be at the proliferator-activated receptor-gamma [14].

The aim of this work was to evaluate the possible analgesic activity and the lethal dose 50 ($LD_{50}$) of four 5-ASA derivatives, two amides (**1** and **2**) and two imides (**3** and **4**), in the mouse model. In addition, the Log P of the compounds and their affinity for COX enzymes were theoretically calculated.

*Address correspondence to this author at the Sección de Estudios de Posgrado e Investigación y Departamento de Bioquímica, Escuela Superior de Medicina, Plan de San Luis y Díaz Mirón, México, D.F., 11340; Fax +52 555 7296000-Ext. 62747; E-mail: jtrujillo@ipn.mx

## CHEMISTRY

The synthesis of the target compounds was carried out by following the reaction sequences outlined in Scheme **1**. Thus, 5-ASA was made to react with maleic or succinic anhydride to give succinamide (**1**) and maleamide (**2**), respectively. They were mixed in tetrahydrofuran (THF) as a solvent. The corresponding amides were heated at 80 °C using sodium acetate as a catalyzing agent and acetic anhydride as a dehydrating agent to obtain the succinimide (**3**) and maleimide (**4**), respectively. Physical and spectral data are listed in the experimental section.

## PHARMACOLOGY

Given that the assayed compounds are structurally related to salicylic acid, they may act on COX enzymes [15,16]. One method to evaluate this possibility is the writhing test by using acetic acid administered intraperitonealy as was reported by the research group of Deraedt [17], a method that we used to evaluate the analgesic effect mediated by COX enzymes. Fig. (**1**) shows that 5-ASA derivatives have analgesic activity with a potency very similar to their starting material, but with an additional maleic or succinic acid fragment that gives them better lipophilicity and thus permits them to cross the lipidic barrier, judging by their Log P values. This is particularly true for the compounds with two rings (**3** and **4**) as can be seen in (Table **1**). Thus, the compounds could cross the lipid barrier to be absorbed in the gastrointestinal system and could act on the COX enzymes that are induced in the peritoneal tissue by acetic acid [17], possibly explain-

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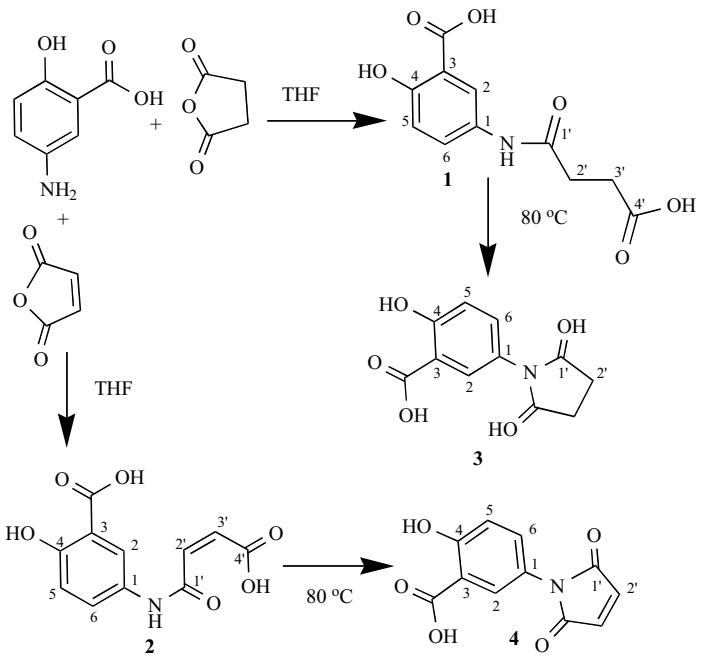


**Scheme 1.** Synthesis of 5-ASA derivatives **1**, **2**, **3** and **4**.

ing the greater affinity of the imides and especially for **3,** which had a dose-dependent analgesic activity (see Fig. **1**). The analgesic activity for all compounds was observed from 0.1 mg/Kg to 16 mg/Kg.

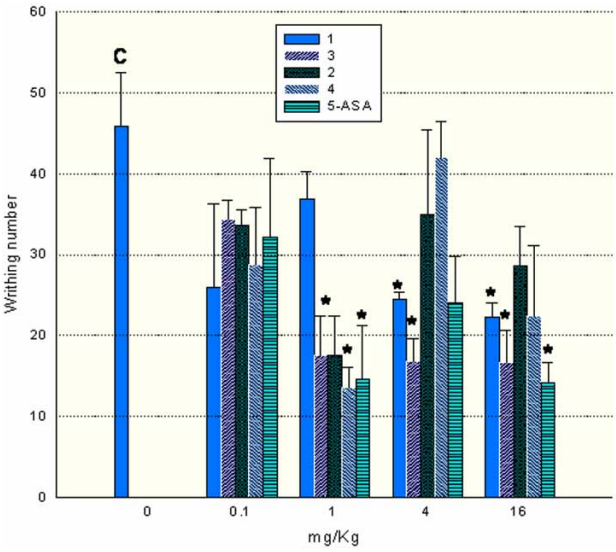


**Fig. (1).** Analgesic effects of 5-ASA and its derivatives at 0.1, 1, 4 and 16 mg/kg during 30 min. "c" is the control whereas that the other bar groups correspond to the compound tested.

In the acute toxicity assays, no deaths were observed after applying 5-ASA derivatives at a dose >1000 mg/kg of body weight (except for **4,** see Table **1**) whereas that $LD_{50}$ for 5-ASA is 469 mg/Kg, being this compound more toxic than its derivatives [2]. Although the mechanism of the side effects of 5-ASA is not clearly understood [7-10], it could be due to its structural relation with anilines, which is metabolized by cytochrome P-450 yielding free radicals [18], and such free radicals damage the cell membranes. Thus, in this study, the amides and imides were synthesized to block the amino group from 5-ASA [19], to decrease the toxic effect.

## MOLECULAR MODELING

5-ASA is a liposoluble compound, which could explain its availability in the upper bowel [20], the Log P of the 5-ASA derivatives was evaluated by theoretical calculations to ensure that they could cross the cell membrane barriers. These results show that 5-ASA is more liposoluble (Log P = 0.64) than amides (**1** and **2**), but lesser than imides (**3** and **4**), which is in agreement with data reported [21]. In spite of the imides being more liposoluble (Log P = 1.70 and 1.92 for **3** and **4**, respectively) than 5-ASA and its amides, this property was not reflected in their analgesic activity (see Fig. **1**). The higher Log P of the imides suggests that they could cross the cell membrane barriers more efficiently, thus enabling them to arrive at the recognition binding site of COX enzymes.

For rational drug design, it is important to know the structure of the target receptor or enzyme, particularly at the recognition binding site. To our knowledge, there have not been any complete studies which indicate that 5-ASA could act as a nonsteroidal anti-inflammatory drug [22]. Therefore, in this study, we explored the binding sites of COX I and II using as ligands 5-ASA and its derivatives by docking simulations as well as by testing the analgesic effects of the compounds by using *in vivo* assays. The results show that 5-ASA and its derivatives can fit in the active site of COX I and II, with affinities very close to μM in both cases being more active on COX II. This binding model indicates that the analgesic effects could be partially caused by the inhibition of COX enzymes [23].

**Table 1. The Theoretical and Experimental Values of 5-ASA and its Derivatives**

| Compounds | [a]Log P | $LD_{50}$ (mg/kg) | [b]$K_d$ (μM) COX II | [b]$K_d$ (μM) COX I | Ratio $K_d$ COXII/COXI |
|---|---|---|---|---|---|
| **5-ASA** | 0.64 | 469* | 10.3 | 4.24 | 2.42 |
| **1** | –0.26 | 2700.89 | 0.284 | 0.64 | 0.34 |
| **2** | –0.57 | 1927.41 | 0.151 | 0.43 | 0.35 |
| **3** | 1.70 | 3934.31 | 0.609 | 1.53 | 0.39 |
| **4** | 1.92 | 261.51 | 0.152 | 4.15 | 0.03 |

*Data taking from Abdel-Alim *et al*., 2005 [2]

[a]Data calculated by www.Logp.com.

[b]Data calculated by AutoDock 3.0

The docking calculations show that the 5-ASA derivatives have more affinity on the COX II enzyme than 5-ASA (Table **1**), which could be due to the fact that 5-ASA derivatives exhibit more hindrance effects, giving them more surface area than 5-ASA. This could allow more interactions on COX II than COX I as a result of the greater surface area in the active site of COX II [15,16].

The results show that in principle all compounds adopt similar binding modes (as can be seen for compounds **2** and **4** in Fig**s. 2** and **3**), in which they recognize the binding site and make contact with several well-known aminoacids that participate in the substrate recognition [24,25].

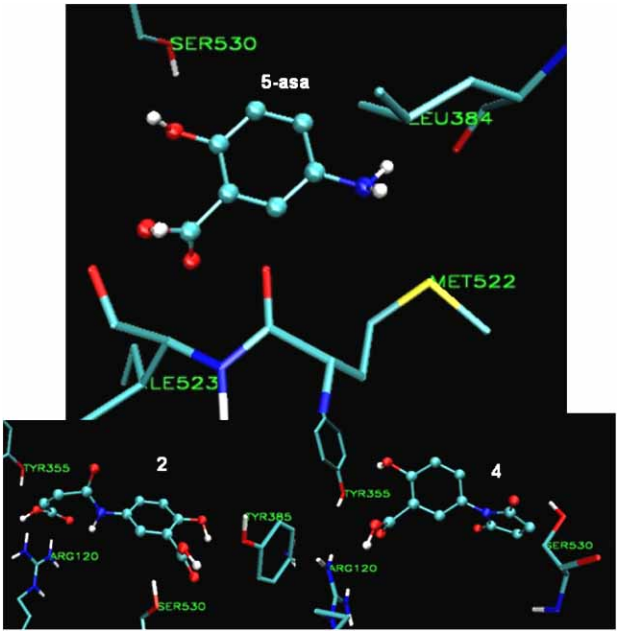


**Fig. (2).** Docking of 5-ASA, **2** and **4** on COX I. The ligands are depicted as CPK and aminoacid residues as bond methods.

The aromatic ring of the compounds is placed near the aromatic residues (Tyr355 and 385) of the enzymes, the latter of which and especially the Phe residues increase the hydrophobic environment and the possibility of making π–π interactions with the aromatic ring of the ligands. The most important interactions are depicted in (Figs. **2** and **3**), such as the π–π interactions formed between the aromatic ring of the compounds and the aromatic residues of the COX enzymes (Tyr385, 355), the van der Waals forces, the hydrophobic interactions and hydrogen bonds between the hydroxyl groups of Ser530 and Tyr385, as well as the interaction of the carboxylic carbonyl group (from aromatic ring) of the compounds and the guanidine group of both Arg513 and 120 making hydrogen bonds [24]. For COX II, the interaction of the amide carbonyl group of the 5-ASA derivatives with Arg120 and Tyr355 is conserved. Interestingly, the amide group of the compounds can interact with Hys90 and Arg513 in the side pocket of COX II [25].

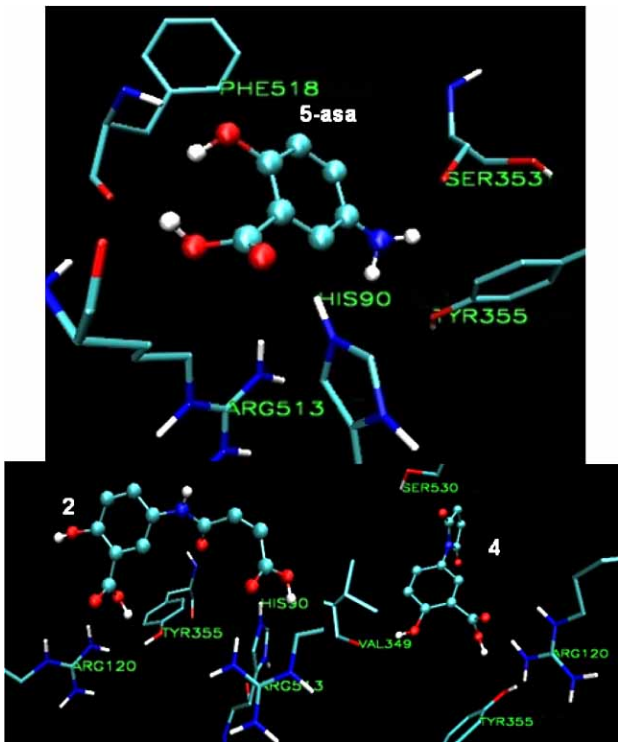


**Fig. (3).** Docking of 5-ASA, **2** and **4** on COX II. The ligands are depicted as CPK and aminoacid residues as bond methods.

The fact that all compounds are completely buried inside the active site of COX might contribute to the stabilization of the enzyme-inhibitor complex, since the aromatic ring moiety of the ligands is rich in π orbitals, which confer upon them the greater possibility of making contact with the aromatic residues (Tyr355, 385). Thus, the main binding recognition between the ligands and the COX enzymes is driven by π–π interactions (see Figs. **2** and **3**). Notable, the α,β-unsaturated carbonyl of the derivatives from maleic anhydride (**4**) could make a 1-4 Michael reaction with the hydroxyl group of Ser530 (see Figs. **2** and **3**), which could result in covalent bond formation and consequently, the inhibition must be irreversible [26]. 5-ASA, although smaller and thinner than its derivatives, displays better π–π interactions with aromatic residues located at the active site. On the other hand, the amide derivatives are close to Tyr355 (Fig. **2**) and display more contacts with this aromatic residue than 5-ASA.

In conclusion, the COX affinity of 5-ASA and its derivatives as well as the results of the writhing test showed that these compounds could have an analgesic activity mediated in part by the inhibition of the COX enzymes.

## EXPERIMENTAL SECTION

### General

Acetic anhydride, dry thetrahydrofuran, sodium bicarbonate, maleic and succinic anhydrides and 5-aminosalicylic acid were purchased from Aldrich. Mice were source from the ESM-IPN Bioterium Department.

IR spectra were recorded on a MIDAC M2000 FT-IR instrument using KB**r.** $^{1}$H and $^{13}$C NMR spectra were recorded on a Jeol GSX-270 spectrometer using DMSO-$d_6$ as solvent and TMS as internal reference. The reactions were monitored by thin-layer chromatography (*TLC*, silica gel 60 $F_{254,}$ 0.25 mm). The developed *TLC* was observed under ultraviolet light (at 254 nm) on ULTRA-LUM equipment.

### Chemistry

#### *Preparation of Amides*

Equimolar quantities of succinic anhydride and 5-ASA were mixed in tetrahydrofuran, at room temperature (see Scheme **1**). The mixture was stirred for 1 h; the reaction was monitored by *TLC* (acetone/ethanol 1:1; $SiO_2$). The synthesized compounds were suspended and washed with $H_2O$ (3x30 mL); the pH was reached at ≅ 4 by using HCl. They were filtered and then dried at 40 °C for 24 h.

#### *4-hydroxy-3-carboxy-N-butanoic Acid (1)*

A mixture of 5-ASA (2 g, 14.29 mmol) and succinic anhydride (1.3 g, 14.29 mmol) in tetrahydrofuran was stirred for 1 h. Then, the organic phase was evaporated and the product was washed with $H_2O$, filtered and dried for 24 h at 40 °C. The product was obtained in 95% yield as a white solid; m.**p. 2**08.5 ºC; $^{1}$H NMR (300 MHz, DMSO-$_{d6}$): δ/ppm 2.39 (2H, s, H-2'), 2.49 (2H, s, H-3'), 6.93 (1H, d, *J*=7.2, H-5), 7.63 (1H, d, *J*=7.2, H-6), 8.11 (1H, s, H-2); $^{13}$C NMR (75.4 MHz, DMSO-$d_6$): δ/ppm 117.39 (C-5), 117.96 (C-3), 120.92 (C-2), 127.39 (C-2'), 127.59 (C-6), 131.59 (C-1), 131.59 (C-3'), 155.94 (C-4), 157.60 (C-1'), 174.53 (C-4').

#### *4-hydroxy-3-carboxy-N-oxo-(Z)-2-butenoic Acid (2)*

Compound **2** was synthesized under the same procedure for **1**, but maleic anhydride replaced succinic anhydride. The product was obtained in 91% yield as a yellow solid; m.p.: 226 ºC; $^{1}$H NMR (300 MHz, DMSO-$d_6$): δ/pmm 6.31 (1H, d, *J*=12.06, H-3') 6.48 (1H, d, *J*=12.06, H-2'), 6.96 (1H, d, *J*=8.91, H-5), 7.71 (1H, d, *J*=6.6, H-6), 8.19 (1H, s, H-2); $^{13}$C NMR (75.4 MHz, DMSO-$d_6$) δ/pmm 112.7 (C-3), 117.3 (C-5), 121.0 (C-2), 127.5 (C-6), 130.0 (C-1), 130.5 (C-3'), 131.6 (C-2'), 157.6 (C-4), 163.0 (C-1'), 166.7 (C-4').

#### *Preparation of Imides*

The starting materials **1** and **2** were transformed to the corresponding *N*-arylimides (**3** and **4**) by heating them for 3 h at 80-85°C in the presence of sodium acetate (0.29 mmol) as a catalyzing agent and acetic anhydride (5 mL) as a dehydrating agent. These reactions were monitored by *TLC* (acetone/ethanol 1:1, $SiO_2$). The resulting mixtures were cooled, and the products were washed with $H_2O$ (3x30 mL) until obtaining a pH ≅ 3.

#### *5-(2,5-dioxo-pyrrolidin-1-yl)-2-hydroxy-benzoic Acid (3)*

**1** (2g, 15 mmol) and sodium acetate (15 mmol) were mixed in equimolar quantities using acetic anhydride as a solvent and as dehydrating agent. The solution was mixed and stirred for 1 h. Then, the organic phase was evaporated under reduced pressure and the product was washed with $H_2O$, filtered and dried for 24 h at 40 °C. The product was obtained in 52% yield as a white solid; m.p.: 174 ºC; $^{1}$H NMR (300 MHz, DMSO-$d_6$): δ/ppm 3.42 (4H, m, *J*=6.99, H-2',3'), 7.19 (1H, d, *J*=7.2 H-6), 7.61 (1H, d, *J*=7.2, H-2), 7.61 (1H, d, *J*=7.2, H-5); $^{13}$C NMR (75.4 MHz, $DMSO_4$-$d_6$) δ/ppm 28.92 (C-2'), 117.29(C-3), 123.00 (C-1), 123.00 (C-5), 132.83 (C-2), 162.14 (C-4), 177.76 (C-1').

#### *4-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-2-hydroxy-benzoic Acid (4)*

Compound **4** was synthesized from **2,** following the same procedure described above for the preparation of **3**. The product was obtained in 98% yield as a brown solid; m.p.: 229 ºC; $^{1}$H NMR (300 MHz, DMSO-$_{6}$): δ/pmm 7.19 (1H, s, H-2), 7.30 (1H, d, *J*=8.7, H-5), 7.58 (1H, d, *J*=8.7, H-6), 7.78 (2H, d, *J*=2.1, H-2',3'); $^{13}$C NMR (75.4 MHz, DMSO-$d_6$): δ/pmm 113.2(C-3), 117.7 (C-5), 122.8 (C-1), 128.8 (C-2), 134.3 (C-6), 134.6 (C-2'), 160.4 (C-4), 170.1 (C-1').

The structure of the compounds was characterized by $^{1}$H and $^{13}$C NMR analysis and is in agreement with the literature [19].

### Molecular Modeling

The Log P values of 5-ASA and its derivatives were predicted by using Log P software available on the website (http://www.logp.com) which has been used successfully [27].

To explore the affinity of the compounds for COX enzymes (I and II) and also to know which amino acids are involved in the ligand recognitions, docking simulations were done based on the crystal structure of these enzymes. They have the following PDB code: 1PGE for COXI and 5COX for COXII [15,16].

The three-dimensional minimum energy structure of the ligands was obtained by means of the HF/6-31G* level by using the Gaussian program [28]. The partial atomic charges of the ligands were assigned by using the Gasteiger-Marsili formalism and all possible rotable bonds were assigned by using the AutodockTools, a program included in Autodock. Then, the enzymes were prepared by using the same software. Finally, the ligands were docked at the active site of both COX enzymes by using the Autodock software version 3.0.5 [29]. All docking simulations were carried out by using the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals and a maximum number of energy evaluations ($1.0 \times 10^7$). The resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings.

### Pharmacology

The male CD1 mice (20-25 g) used for the test were housed in standard cages (5 per cage) at room temperature (20±5 °C) with both food and water *ad libitum*. All pharmacological tests were carry out after be approved by local committed care and used animals (ESM-IPN-Mexico City).

All compounds were administered at different doses (0.1, 1, 4 and 16 mg/kg); 5-ASA was used as positive control. They were suspended in vegetable oil, which was used as a vehicle; the compounds or vehicle were administered orally 30 min before all experiments.

#### *Analgesic Activity*

This test was carried out by using the writhing test method described by Koster *et al*., [30]. The writhes were induced by intraperitoneal injection of 0.4% acetic acid (v/v) into a group of n = 5. The number of muscular contractions was counted for 30 min after acetic acid injection. The data represent the average of the total number of writhes observed during 30 min (see Fig. **1**).

#### *Acute Toxicity*

The mean lethal dose ($LD_{50}$) was determined for 5-ASA derivatives in male CD1 mice weighting 22.5 ± 2.5 g. Each 5-ASA derivative was applied intraperitoneally, using vegetable oil as a vehicle (the hundredth part of the animal weight). The behavior of the animals after administration of the compounds was observed during the first 2 h and the number of deaths was registered at 24 h [31].

### Statistical Analysis

Data were analyzed by using the Newman-Keulds multiple comparison test (control responses and drug responses). A *P* value of less than 0.05 was considered significant. Statistical analysis was conducted by using the Sigma Stat software.

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