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# Pharmacokinetics of Anti-SARS-CoV Agent Niclosamide and Its Analogs in Rats

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## ABSTRACT

Niclosamide has been demonstrated with inhibitory activity on the replication of SARS-CoV in Vero E6 cells. This study examined the pharmacokinetics and oral bioavailability of niclosamide and its two analogs, BPR1H366 and BPR1H369, in male Sprague-Dawley rats. After a single 2-mg/kg intravenous dose, the total body clearance (CL) of niclosamide, BPR1H366 and BPR1H369 was 20.0 ± 2.9, 26.7 ± 4.4 and 39.4 ± 6.7 mL/kg/min, and the volume of distribution at steady state ($V_{SS}$) was 0.9 ± 0.4, 0.3 ± 0.1 and 1.1 ± 0.2 L/kg, respectively. The half-life ($t_{1/2}$) of BPR1H366 and BPR1H369 was 2.6 ± 0.3 and 3.7 ± 1.1 hr, respectively, shorter than 6.7 ± 2.0 hr of niclosamide. The AUC was 1413 ± 118, 1019 ± 203 and 750 ± 113 ng/mL × hr for niclosamide, BPR1H366 and BPR1H369, respectively. After a single 5-mg/kg oral dose, all three compounds were rapidly absorbed. Niclosamide showed the highest $C_{max}$ of 354 ± 152 ng/mL within 30 min after oral gavage. The oral bioavailability of niclosamide, BPR1H366 and BPR1H369 was 10%, 12% and 15%, respectively. Our results demonstrated that the extents of drug exposure of the three compounds were comparable in rats. The pharmacokinetic properties of the compounds in humans are needed to be determined before their potential uses in SARS-CoV infected patients.

Key words: SARS, Coronavirus, Niclosamide, Pharmacokinetics, Bioavailability.

## INTRODUCTION

Severe Acute Respiratory Syndrome (SARS) is a respiratory illness caused by the infection of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV)[(1-2)]. The major symptoms of SARS are hyperpyrexia, chilling, cough and dyspnea. This disease emerged in several countries during the period of November 2002 to June 2003. World Health Organization (WHO) reported a total of 8096 SARS patients that were accompanied by either pneumonia or respiratory distress syndrome worldwide and of these 774 patients died[(3)]. Neither antiviral therapy nor vaccine is currently available. The outbreak of SARS pandemic has led to the search for active antiviral compounds to treat the disease.

Niclosamide [5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide] is an effective anthelmintic drug for the treatment of intestinal cestode in fections in humans and animals in many countries for a few decades[(4-5)]. It is also used as a molluscicide for treating water in the control of schistosomiasis[(6-7)]. A previous study reported that niclosamide exhibited a concentration-dependent inhibitory effect on the replication of SARS-CoV in Vero E6 cells[(8)]. An *in silico* binding study used the crystal structure of the SARS-CoV main proteinase, 3CLpro, a key enzyme for the proteolytic processing of the replicase polyproteins 1a and 1ab, demonstrated the binding of niclosamide to the protein, and suggested niclosamide as a template for designing SARS-CoV proteinase inhibitors[(9)]. Niclosamide is active against the SARS-CoV in the micromolar levels as previously reported[(8)], it serves as a validated drug lead from which active anti-SARS-CoV analogs with optimal pharmacokinetic properties may be designed. Two niclosamide analogs, BPR1H366 [5-chloro-*N*-(2, 4-dichloro)-2-hydroxybenzamide] and BPR1H369 [5-chloro-*N*-(2-chloro-4-trifluoromethyl)-2-hydroxybenzamide], were designed and synthesized as shown in Figure 1. The purpose of this study was to determine the pharmacokinetic parameters and the oral bioavailability of niclosamide and its two chemical analogs in rats.

## MATERIALS

### I. *Chemicals*

Niclosamide (Lot No. 047H0703) and naproxen (Lot No. 02230LB), as the internal standard, were purchased from Sigma-Aldrich Corp. (St. Louis, Missouri, USA). BPR1H366 and BPR1H369 were synthesized and provided by H. P. Hsieh of the Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli, Taiwan, ROC. The chemical purity for both niclosamide analogs was greater than 99%. All of the

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other chemicals were of reagent grade and were obtained commercially.

II. *Animal Study*

Male Sprague-Dawley rats of 330-380g were obtained from BioLasco, Taiwan Co., Ilan, Taiwan. Animals were surgically implanted with a jugular-vein cannula one day before dosing and fasted for 12 hr prior to oral dosing. The compounds were administered intravenously (i.v.) and orally (p.o.) at a dose of 2-mg/kg and 5-mg/kg, respectively. The dosing solutions were prepared by dissolving the compound in a mixture of dimethyl sulfoxide (DMSO)/cremophor EL/water (3/15/82 v/v/v). The dosing solutions were administered intravenously via the tail vein or orally gavaged. Blood samples of 150 µL were collected via the jugular-vein cannula at 0 (immediately before dosing), 2, 5 (iv only), 15 and 30 min and at 1, 2, 4, 6, 8, 12 and 24 hr after compound administration into EDTA-containing tubes and kept on ice. Blood samples from rats receiving no compounds were also collected for blanks and calibration curves. Immediately after collecting the blood sample, 150 µL of physiological saline (containing 30 units of heparin per mL) was injected to the rat via the jugular-vein cannula. Plasma samples were then collected by centrifugation (1500 ×g for 15 min at 4°C) with a Beckman Model Allegra$^{TM}$ 6R centrifuge and stored at –20°C until LC-MS/MS analysis. The use of the animals was approved by the institutional animal care and use committee of National Health Research Institutes.

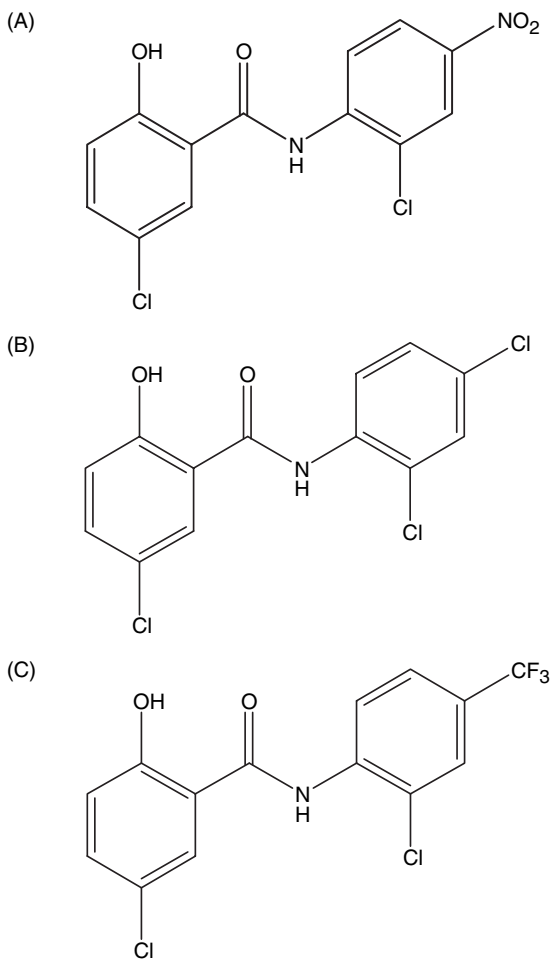


**Figure 1.** Chemical structure of (A) Niclosamide, (B) BPR1H366 and (C) BPR1H369.

III. *Plasma Sample Preparation*

Plasma (30 µL) was mixed with 60 µL of acetonitrile containing 250 ng/mL of naproxen as the internal standard (IS). The mixture was vortexed for 30 sec and then centrifuged at 21,000 ×g for 20 min in an Eppendorff centrifuge Model 5417c at room temperature. An aliquot (10 µL) of the supernatant was injected onto LC-MS/MS. To prepare the samples of the calibration curve, the blank plasma (30 µL) containing various concentrations of niclosamide or its analogs was mixed with 60 µL of acetonitrile containing the IS (250 ng/mL).

IV. *LC-MS/MS Analysis*

The HPLC system consisted of an Agilent 1100 series LC System with two pumps (Palo Alto, CA, USA) and an Xterra MS-$C_{18}$ reverse-phase column (5 µm, 2.1 × 20 mm) interfaced to an API 3000$^{TM}$ tandem mass spectrometer equipped with a TurboIonSpray in the negative ion mode (Applied Biosystems, Foster City, CA, USA). Mobile phase consisted of 10 mM ammonium acetate containing 0.1% of formic acid (Solvent A) and acetonitrile (Solvent B). The following stepwise gradient system was used: 70% A to 2% A (0-0.5 min), 2% A (0.5-4.0 min), 2% A to 70% A (4.0-4.5 min), 70% A (4.5-7.0 min). Total running time was 7 min. The retention times of niclosamide, BPR1H366, BPR1H369 and naproxen were 1.9, 2.1, 2.1 and 1.5 min, respectively. Data acquisition was via multiple reaction monitoring (MRN). The collision energy was -40V for the analyte and -28V for IS. The ions monitored (m/z) for niclosamide, BPR1H366, BPR1H369 and naproxen were 327.0/172.9, 315.9/280.1, 348.0/311.9 and 229.2/184.9, respectively. A good relationship observed over the concentration range of 0.5-1000 ng/mL was fitted using a quadratic regression mode and weighted by reciprocal concentration (1 / x) ($r$ > 0.9983). The intra- and inter-day accuracy ranged from 93 to 114% (n = 5). Intra- and inter-day deviation of precision were all below 7% (n = 5). The lower limit of quantitation (LLOQ) was 0.5 ng/mL. Plasma samples that had concentrations above the upper limit of quantitation (1000 ng/mL) were diluted proportionally with control plasma prior to extraction with acetonitrile.

V. *Data Analysis*

Plasma concentration data were analyzed using standard non-compartmental method with WinNonLin

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**Table 1.** Plasma concentrations of niclosamide, BPR1H366 and BPR1H369 after a single 2-mg/kg intravenous dose to rats. (n = 3 per time point, mean ± SD)

| Time | Concentration (ng/mL) | | |
|---|---|---|---|
| | Niclosamide | BPR1H366 | BPR1H369 |
| 2 min | 8150 ± 1294 | 7783 ± 1531 | 5060 ± 707 |
| 5 min | 3563 ± 1096 | 1620 ± 471 | 1703 ± 142 |
| 15 min | 948 ± 306 | 280 ± 78 | 357 ± 64 |
| 30 min | 205 ± 23 | 69.5 ± 32.6 | 111 ± 15 |
| 1 hr | 56.6 ± 19.6 | 10.0 ± 3.5 | 23.3 ± 7.0 |
| 2 hr | 38.3 ± 27.0 | 11.9 ± 13.4 | 12.1 ± 9.0 |
| 4 hr | 11.2 ± 2.9 | 2.9 ± 1.0 | 6.0 ± 4.8 |
| 6 hr | 6.8 ± 3.8 | 2.0 ± 0.4 | 3.3 ± 1.5 |
| 8 hr | 6.3 ± 5.6 | 1.1 ± 0.4 | 2.0 ± 0.9 |
| 12 hr | 2.4 ± 0.6 | blq | 1.0 ± 0.6 |
| 24 hr | blq | blq | blq |

blq: below the limit of quantitation (0.5 ng/mL)

**Table 2.** Plasma concentrations of niclosamide, BPR1H366 and BPR1H369 after a single 5-mg/kg oral dose to rats. (n = 3 per time point, mean ± SD)

| Time | Concentration (ng/mL) | | |
|---|---|---|---|
| | Niclosamide | BPR1H366 | BPR1H369 |
| 15 min | 340 ± 161 | 268 ± 146 | 98 ± 15 |
| 30 min | 354 ± 152 | 107 ± 50 | 118 ± 19 |
| 1 hr | 83.6 ± 31.7 | 31.6 ± 16.9 | 42.3 ± 18.6 |
| 2 hr | 37.5 ± 10.6 | 71.1 ± 55.9 | 5.0 ± 3.1 |
| 4 hr | 12.7 ± 11.9 | 22.4 ± 6.8 | 19.0 ± 5.2 |
| 6 hr | 7.5 ± 3.5 | 14.4 ± 3.9 | 29.7 ± 8.6 |
| 8 hr | 5.1 ± 2.9 | 9.3 ± 4.2 | 13.3 ± 2.4 |
| 12 hr | 3.8 ± 2.0 | 3.2 ± 1.3 | 13.1 ± 8.9 |
| 24 hr | blq | blq | blq |

blq: below the limit of quantitation (0.5 ng/mL)

software program from Pharsight Corp, (Mountain View, CA, USA). The peak plasma concentration ($C_{max}$) and the time to reach $C_{max}$ ($T_{max}$) were determined from the actual data obtained after intravenous or oral administration. The terminal elimination rate constant ($\lambda$) was calculated by fitting individual data for three or more terminal points of the plasma concentration profile with a log-linear regression equation using the least-squares method. The corresponding elimination half-life ($t_{1/2}$) was calculated by dividing ln2 by $\lambda$. The area under the plasma concentration-time curve from zero to infinity ($AUC_{0\text{-inf}}$) was calculated using the trapezoidal rule with extrapolation to infinity. The absolute bioavailability (F, %) after oral administration was estimated as $F(\%) = (AUC_{po} \times Dose_{iv})/(AUC_{iv} \times Dose_{po}) \times 100$.

## RESULTS AND DISCUSSION

Niclosamide is one of the halogenated salicylanilides, which are highly hydrophobic having a very low aqueous solubility[10]. The aqueous solubility of its two analogs, BPR1H366 and BPR1H369, is also low at 0.23 and 0.42 ng/mL, respectively. (in-house data). In this study, these three compounds were dissolved in a mixture of DMSO/cremophor EL/water (3/15/82 v/v/v) at a concentration of 2 mg/mL. These formulated true solutions stay physically and chemically stable within 24 hr after preparation and before dosing. The plasma concentrations of compounds after single intravenous and oral administration in rats were listed in Tables 1 and 2. Figures 2 and 3 illustrate the plasma concentration verses time after the compounds given intravenously and orally in rats, respectively. The compounds in the rat plasma were measurable up to 12 hr after dosing for niclosamide (2.4 ± 0.6 ng/mL) and BPR1H369 (1.0 ± 0.6 ng/mL), and 8 hr for BPR1H366 (1.1 ± 0.4 ng/mL). Table 3 summarizes the pharmacokinetic parameters of the three compounds in the two dosing routes. The total body clearance (CL) of niclosamide, BPR1H366 and BPR1H369 was 20.0 ± 2.9, 26.7 ± 4.4 and

**Table 3.** Pharmacokinetic parameters of niclosamide, BPR1H366 and BPR1H369 after a single 2-mg/kg intravenous and a 5-mg/kg oral administration in rats.

| Compound | Route | Dose (mg/kg) | $t_{1/2}$ (hr) | CL (mL/min/kg) | $V_{ss}$ (L/kg) | $C_{max}$ (ng/mL) | $T_{max}$ (hr) | $AUC_{(0\text{-inf.})}$ (ng/mL × hr) | F (%) |
|---|---|---|---|---|---|---|---|---|---|
| Niclosamide | i.v. | 2 | 6.7 ± 2.0 | 20.0 ± 2.9 | 0.9 ± 0.4 | - | - | 1413 ± 118 | - |
| BPR1H366 | i.v. | 2 | 2.6 ± 0.3 | 26.7 ± 4.4 | 0.3 ± 0.1 | - | - | 1019 ± 203 | - |
| BPR1H369 | i.v. | 2 | 3.7 ± 1.1 | 39.4 ± 6.7 | 1.1 ± 0.2 | - | - | 750 ± 113 | - |
| Niclosamide | p.o. | 5 | 6.0 ± 0.8 | - | - | 354 ± 152 | 0.4 ± 0.1 | 429 ± 100 | 10 |
| BPR1H366 | p.o. | 5 | 2.9 ± 1.0 | - | - | 268 ± 146 | 0.3 ± 0.0 | 360 ± 66 | 12 |
| BPR1H369 | p.o. | 5 | 3.3 ± 1.7 | - | - | 118 ± 18 | 0.4 ± 0.1 | 342 ± 71 | 15 |

CL: clearance; $V_{ss}$: volume of distribution at steady state; $t_{1/2}$: elimination half-life; AUC: area under the concentration-time curve; $C_{max}$: peak concentration; $T_{max}$: time to reach $C_{max}$; F: absolute bioavailability.

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39.4 ± 6.7 mL/kg/min, respectively, suggesting moderate clearance of these three compounds[11]. The volume of distribution at steady state ($V_{ss}$) of niclosamide, BPR1H366 and BPR1H369 was 0.9 ± 0.4, 0.3 ± 0.1 and 1.1 ± 0.2 L/kg, respectively. The $V_{ss}$ of BPR1H366 was less than the total body water in rats (~0.7 L/kg)[11], suggesting that BPR1H366 was extracellularly distributed mainly in the blood circulation. Niclosamide showed a longer $t_{1/2}$ (6.7 ± 2.0 hr) than that of BPR1H366 (2.6 ± 0.3 hr) and BPR1H369 (3.7 ± 1.1 hr). Niclosamide exhibited the highest AUC among the three compounds.

After an oral dose of 5-mg/kg, plasma concentration of niclosamide, BPR1H366 and BPR1H369 was measurable up to 12 hr at 3.8 ± 2.0, 3.2 ± 1.3 and 13.1 ± 8.9 ng/mL, respectively (Table 2). As shown in Figure 3, all three compounds were rapidly absorbed with a $T_{max}$ less than 30 min after oral administration. Among them, niclosamide showed the highest $C_{max}$ at 354 ± 152 ng/mL, followed by BPR1H366 at 268 ± 146 ng/mL, and BPR1H369 at 118 ± 18 ng/mL. The concentration-time profiles revealed a second peak at 2 hr for BPR1H366 and at 6 hr for BPR1H369. Schaiquevich *et al*. proposed two mechanisms for two peaks in a concentration-time profile: enterohepatic recirculation and the existence of multiple sites of absorption along the gastrointestinal tract[12]. Further pharmacokinetic studies of these two compounds should be performed to elucidate this phenomenon. Niclosamide exhibited a higher AUC than the two analogs in both intravenous and oral dosing routes, whereas the calculated oral bioavailability of BPR1H369 was the highest among the three showing a poor absorption at only 15%. The oral bioavailability of niclosamide was 10%, which is in agreement with the previously reported in humans[13]. In addition, an acute toxicology study reported an oral $LD_{50}$ of niclosamide of > 5000 mg/kg in rats and the no-observed adverse effect level of niclosamide was 2000 mg/kg/day of repeated daily oral administrations in a 4-week subacute toxicology study[10]. These high toxicological doses demonstrated a good therapeutic index and thus a large safety margin for niclosamide. All these results, on the other hand, indicated a major fraction of the orally administered niclosamide molecules retain within the gastrointestinal tract and, therefore, better target the parasites reside in the intestines as it has been an effective anthelmintics against the intestinal cestode infections in humans and animals. Although niclosamide has been used in humans for decades, very little of its pharmacokinetic data was reported in rats. Our results suggested that the anti-SARS-CoV agent niclosamide and its two analogs showed a low oral bioavailability in rats. Nonetheless, the wide safety margin of niclosamide proves itself as a promising drug for treating SARS-CoV infections if a linearity of pharmacokinetics observes within the dose range (5 to 2000 mg/kg, p.o.) reported. Whether the two analogs exhibit the same pharmacokinetic properties in humans as that in rats remains to be explored. Efforts on optimizing formulations that increase oral absorption and on delivery to the SARS-CoV-infected lungs of these compounds are also warranted.

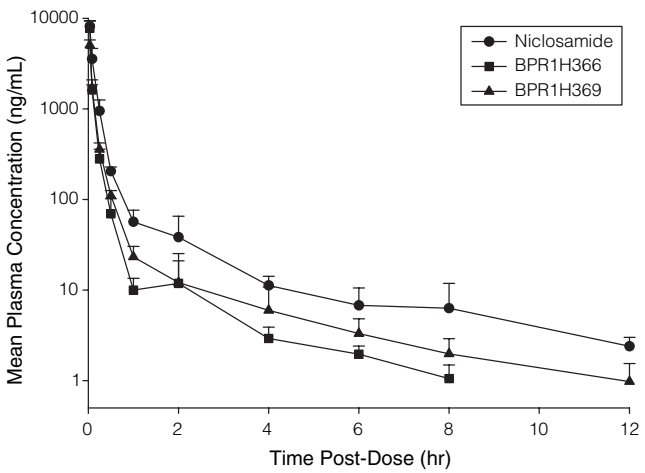


**Figure 2.** Plasma concentrations of niclosamide, BPR1H366 and BPR1H369 after a single intravenous administration of 2-mg/kg to rats (n = 3 per time point, mean ± SD).

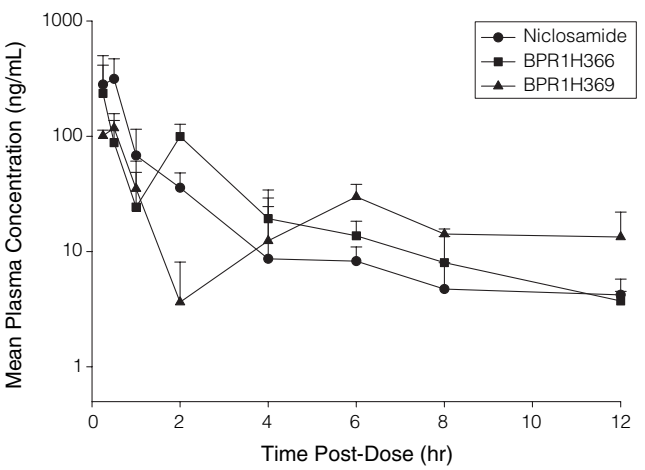


**Figure 3.** Plasma concentrations of niclosamide, BPR1H366 and BPR1H369 after a single oral administration of 5-mg/kg to rats (n = 3 per time point, mean ± SD).

Porcine transmissible gastroenteritis virus (TGEV) shares a similar 3-dimensional crystal protein structure with SARS-CoV and has been utilized as a template *in silico* study searching for SARS-CoV inhibitors[14-16]. We also found that niclosamide and the two analogs inhibited the in vitro replication of TGEV in Swine Testis CRL-1763 cells (ST cells) with an $IC_{50}$ for niclosamide, BPR1H366, and BPR1H369 of 15, 3, and 2 μM, respectively (unpublished results). In addition to the activity against SARS-CoV, the three compounds may also be used to treat porcine TGEV infections. TGEV causes 65% of infectious piglet diarrhea and results in high mortality in porcine neonates in Taiwan[17-18]. The cease of the SARS-CoV pandemic in 2003 relieves the immediate pressure needed to search for effective therapeutics for SARS-CoV infec-

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tions. Nevertheless, niclosamide against SARS-CoV and TGEV as well as its two analogs against TGEV may be reserved as a pool of backup active compounds for treating SARS-CoV infection in humans.

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## REFERENCES

1. Ksiazek, T. G., Erdman, D., Goldsmith, C., Zaki, S. R., Peret, T. and Emery, S. 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. 348: 1953-1966.
2. Drosten, C., Gunther, S., Preiser, W., van der Werf, S., Brodt, H. R., Becker, S., Rabenau, H., Panning, M., Kolesnikova, L., Fouchier, R. A., Berger, A., Burguiere, A. M., Cinatl, J., Eickmann, M., Escriou, N., Grywna, K., Kramme, S., Manuguerra, J. C., Muller, S., Rickerts, V., Sturmer, M., Vieth, S., Klenk, H. D., Osterhaus, A. D., Schmitz, H. and Doerr, H. W. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. 348: 1967-1976.
3. World Health Organization. 21 April 2004, posting date. WHO [Online] http://www.who.int/csr/sars/country/table2004_04_21/en/index.html.
4. Reynolds, J. E. F. 1989. In "Martindale: The Extra Pharmacopoeia". 29th ed. pp. 987-991. Reynolds, J. E. F. ed., The Pharmaceutical Press, London, UK.
5. Katz, M. 1986. Anthelmintics. Current concepts in the treatment of helminthic infections. Drugs 32(4): 358-371.
6. Goldsmith, R. S. 1984. In "Basic and Clinical Pharmacology" pp. 659-660: Katzung, B. G. ed. Lange Medical, Los Angeles, U.S.A.
7. Lowe, D., Xi, J., Meng, X., Wu, Z., Qiu, D. and Spear, R. 2005. Transport of *Schistosoma japonicum cercariae* and the feasibility of niclosamide for cercariae control. Parasitol. Int. 54(1): 83-89.
8. Wu, C. J., Jan, J. T., Chen, C. M., Hsieh, H. P., Hwang, D. R., Liu, H. W., Liu, C. Y., Huang, H. W., Chen, S. C., Hong, C. F., Lin, R. K., Chao, Y. S. and Hsu, T. A. 2004. Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob. Agents Chemother. 48: 2693-2696.
9. Zhang, X. U. and Yap, Y. L. 2004. Old drugs as lead compounds for a new disease? Binding analysis of SARS coronavirus main proteinase with HIV, psychotic and parasite drugs. Bioorg. Med. Chem. 12: 2517-2521.
10. World Health Organization. 2002. WHO [Online] http://www.who.int/whopes/quality/en/Niclosamide.pdf.
11. Davies, B. and Morris, T. 1993. Physiological parameters in laboratory animals and humans. Pharm. Res. 10: 1093-1095.
12. Schaiquevich, P., Niselman, A. and Rubio, M. 2002. Comparison of two compartmental models for describing ranitidine's plasmatic profiles. Pharmacol. Res. 45: 399-405.
13. Campbell, W. C. and Rew, R. S. 1986. Chemotherapy of parasitic disease, Pleum Press, New York, U.S.A.
14. Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R. and Hilgenfeld, R. 2003. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science 300(5626): 1763-1767.
15. Xiong, B., Gui, C. S., Xu, X. Y., Luo, C., Chen, J., Luo, H. B., Chen, L. L., Li, G. W., Sun, T., Yu, C. Y., Yue, L. D., Duan, W. H., Shen, J. K., Qin, L., Shi, T. L., Li, Y. X., Chen, K. X., Luo, X. M., Shen, X., Shen, J. H. and Jiang, H. L. 2003. A 3D model of SARS_CoV 3CL proteinase and its inhibitors design by virtual screening. Acta Pharmacol. Sin. 24(6): 497-504.
16. Jenwitheesuk E. and Samudrala, R. 2003. Identifying inhibitors of the SARS coronavirus proteinase. Bioorg. Med. Chem. Lett. 13(22): 3989-3892.
17. Chen, C. M., Pocock, D. H. and Britton, P. 1993. Genomic organisation of a virulent Taiwanese strain of transmissible gastroenteritis virus. Adv. Exp. Med. Biol. 42: 23-28.
18. Chen, C. M., Cavanagh, D. and Britton, P. 1995. Cloning and sequencing of a 8.4-kb region from the 3'-end of a Taiwanese virulent isolate of the coronavirus transmissible gastroenteritis virus. Virus Res. 38(1): 83-89.
19. Swan, G. E. 1999. The pharmacology of halogenated salicylanilides and their anthelmintic use in animals. J. S. Afr. Vet. Ass. 70(2): 61-70.