The Binding Interactions of the Macrolide Endectocide Ivermectin with the Antibiotics Ampicillin, Chloramphenicol and Tetracycline HCl

M. KANDEEL*[,1], W. ELGAZAR[2] AND Y. KITADE[1,3]

Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelshikh University, Kafrelshikh-33516, Egypt, [1]United Graduate School of Drug Discovery and Medical Information Sciences,Gifu University, Yanagido 1-1, Gifu 501-1193, Japan,[2]Kafrelshikh University Veterinary Hospital, Faculty of Veterinary Medicine, Kafrelshikh University, Kafrelshikh-33516, Egypt, [3]Department of Biomolecular Science,Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan

Ivermectin, chloramphenicol, ampicillin and tetracycline HCl are common drugs in human and veterinary practice. The purpose of this study is to investigate the possible binding interactions between ivermectin and the antibiotics chloramphenicol, ampicillin and tetracycline HCl. Isothermal titration calorimetry was used to determine the binding interactions between ivermectin and these antibiotics. Results indicated that, about three molecules of ampicillin can bind to one molecule of ivermectin and about one molecule of chloramphenicol with one molecule of ivermectin. However, no binding stoichiometry can be detected with tetracycline HCl-ivermectin titration. Furthermore, the binding interactions were accompanied by various biophysical and biochemical mechanisms. This is the first report of such interactions of ivermectin with chloramphenicol, ampicillin and tetracycline HCl. There are possible binding interactions of ivermectin with chloramphenicol and ampicillin. Further studies are required for detecting the impact of this binding on biological aspects of drug actions.

Key words: Ampicillin, binding, chloramphenicol, ivermectin, tetracycline HCl

Ivermectin is a macrocyclic lactone produced by *Streptomyces avermitilis*[1]. It is active at very low dosage against a broad range of internal and external parasites, thus, it is termed a macrolide endectocide. Ivermectin is available for the treatment and control of parasites in cattle, horses, sheep and dogs and cats[2-5]. Recently, ivermectin is used for treating various parasitic infections in humans[6,7].

All members of avermectins are compounds of a complex structure and high molecular weight (MW>850). The chemical structure of ivermectin comprises of pentacyclic lactones, tetrahydrofuran, cyclohexane and two sugar rings (fig. 1). Therefore, owing to the complexity of ivermectin chemical structure and size of the molecule, ivermectins could interact with other compounds, antibiotics and chemotherapeutic agents.

Chloramphenicol, ampicillin and tetracycline HCl are common antibiotics in the veterinary practice. These antibiotics have multiple routes of administration, including topical, oral or parentral routes. Furthermore, their use is approved for wide range of animals, including food and non-food producing animals.

The possibility of combing or contact of ivermectin with chloramphenicol, ampicillin and tetracycline HCl is highly expected in veterinary field remedies. These drugs are characterised by chemical diversity, wide range of applications, several routes of administration and they are administered in most animal species. All these factors created the doubt about the binding of ivermectin with chloramphenicol, ampicillin and tetracycline HCl. If so, the extent this binding, the binding affinities, the number of molecules from each drug involved and mechanisms in these bindings remains unexplored. For this purpose, we carried out an isothermal titration calorimetry (ITC) experiment using ivermectin as receptor drug, which is titrated with ampicillin, chloramphenicol or tetracycline HCl, which are treated as ligands.

ITC measurements were carried out by using VP-ITC (MicroCal Inc., USA). The ivermectin solution in methanol, at a concentration of 100 μM, was loaded into a 1.4 ml sample cell and titrated with the either of ampicillin, tetracycline HCl or chloramphenicol (10-20 mM) in the 250 μl injection

***Address for correspondence**
E-mail: mahmoud.kandeal@vet.kfs.edu.eg

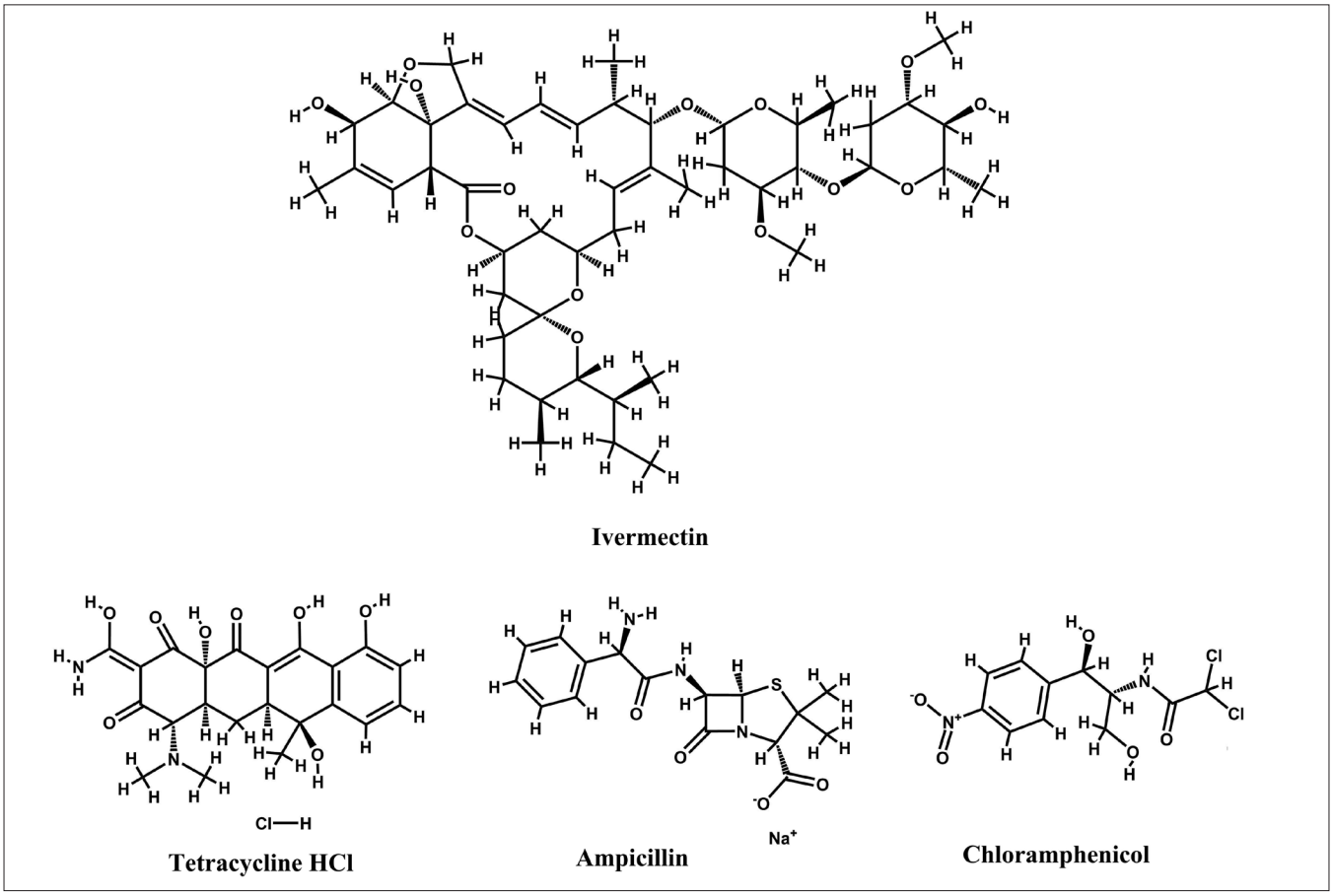

Fig. 1: Chemical structure of ivermectin, ampicillin, chloramphenicol and tetracycline HCl.
The structure of drugs was retrieved from PUBCHEM (http://pubchem.ncbi.nlm.nih.gov/). The structure of drugs was visualised by ChemBioDarw (CambridgeSoft, USA)

syringe. ITC experiments were performed at 30° with a stirring speed 350 rpm and 300 s duration between each 10 µl injection. Control experiments were performed by injecting the antibiotic solutions into methanol to determine the background heats. The apparent heat change after each injection was determined and corrected for heat of dilution of the antibiotics.

The data was fitted to different curve fitting models assuming a single-set of binding sites, two-sets of binding sites or sequential binding sites models. The results includes the estimation of thermodynamic parameters K_a, association constant; ΔH, enthalpy of binding; and n, stoichiometry of binding. The binding affinity is given as dissociation constant ($K_d=1/K_a$). The free energy of binding (ΔG) was calculated from the equations: $\Delta G=RT \ln K_a$ and $\Delta G=\Delta H-T\Delta S$, where ΔS is the entropy changes.

Isothermal titration calorimetry was initially developed for biophysical and thermodynamic analysis of two interacting partners. Nowadays, by improvement of instrument sensitivity, ITC became a method of choice in the broad range of life sciences, particularly in the field of pharmacological and pharmaceutical sciences[8-14]. During addition of ligand, it reacts with the macromolecule resulting in the absorption or release of heat, which can be measured by the microcalorimetry instrument. The produced heat is directly proportional to the fraction of bound ligand as well as to the degree of interaction between the interacting populations. The enthalpy change (ΔH) is the total heat produced or absorbed during titrations. Measurement of heat changes allow for accurate determination of the binding constant, stoichiometry of interaction and complete thermodynamic profile of the ligand–macromolecule interaction.

Figs. 2-4 show typical titration of ivermectin with the antibiotics ampicillin, chloramphenicol and tetracycline HCl. Figs. 2a, 3a and 4a show the raw data heat changes produced by successive injections of ampicillin, chloramphenicol or tetracycline HCl

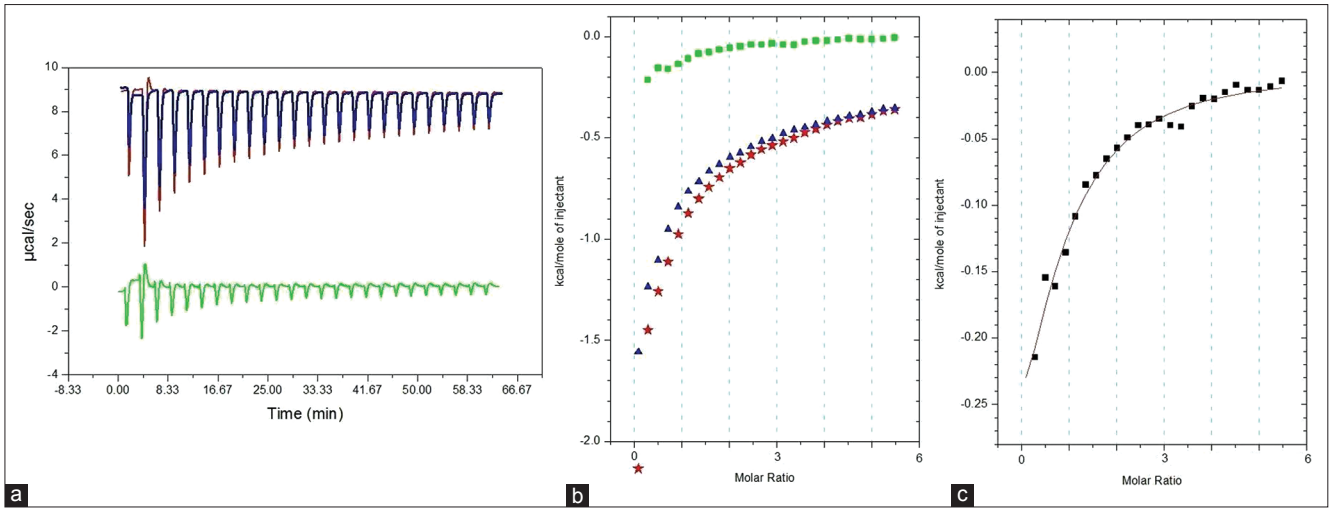

Fig. 2: ITC profiles of the binding of ampicillin with ivermectin.
(a) The raw data heat changes produced by successive injections of ampicillin into ivermectin solution. The raw data of ampicillin-ivermectin titration is represented in red deflection peaks. The raw data of background heat changes are represented in blue peaks. The raw data of background heat changes were subtracted from that ampicillin-ivermectin titration to yield the heat changes of ampicillin-ivermectin binding (green peaks). (b) The binding isotherm of ampicillin-ivermectin binding is represented in red stars, background heat in blue triangles and the integrated binding isotherm of ampicillin-ivermectin binding (after subtraction of background heat) in green rectangles. (c) The integrated binding isotherm of ampicillin-ivermectin interaction

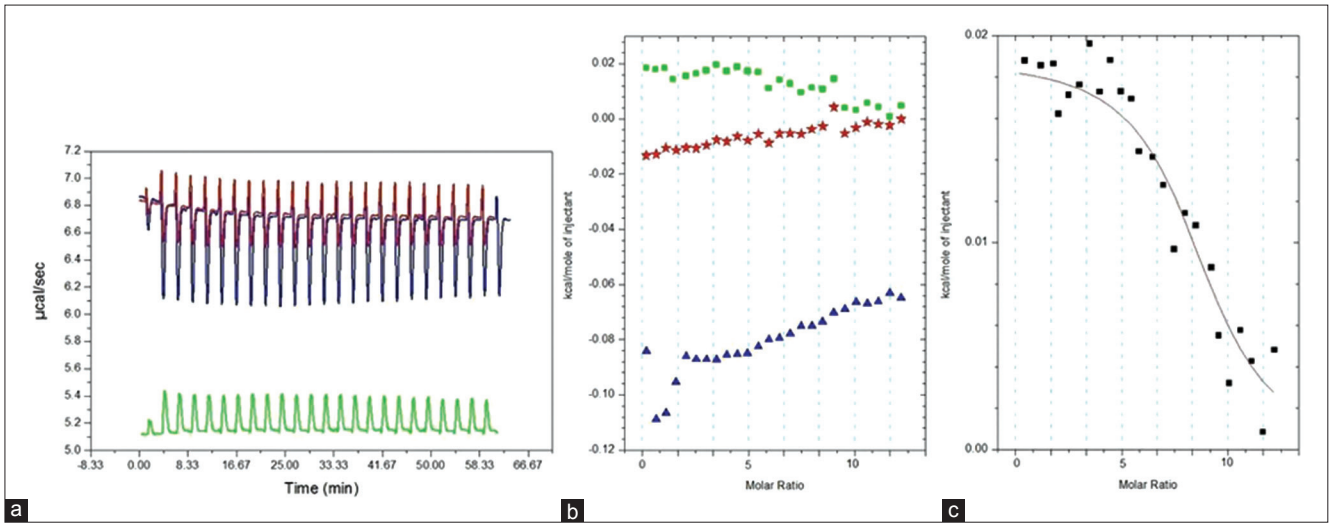

Fig. 3: ITC profiles of the binding of chloramphenicol with ivermectin.
(a) The raw data heat changes produced by successive injections of chloramphenicol into ivermectin solution. The raw data of chloramphenicol-ivermectin titration is represented in red deflection peaks. The raw data of background heat changes are represented in blue peaks. The raw data of background heat changes were subtracted from that chloramphenicol-ivermectin titration to yield the heat changes of chloramphenicol-ivermectin binding (green peaks). (b) The binding isotherm of chloramphenicol-ivermectin binding is represented in red stars, background heat in blue triangles and the integrated binding isotherm of chloramphenicol-ivermectin binding (after subtraction of background heat) in green rectangles. (c) The integrated binding isotherm of chloramphenicol-ivermectin interaction

into ivermectin solution, respectively. Background heat changes were obtained by titration of ampicillin into methanol solution without ivermectin. The raw data of antibiotics-ivermectin titration is represented in red deflection peaks. The raw data of background heat changes (in blue peaks) was aligned with that of antibiotics-ivermectin titration (red peaks). The raw data of back ground heat changes were subtracted from that antibiotics-ivermectin titration to yield the heat changes of antibiotics-ivermectin binding (green peaks). Figs. 2b, 3b, 4b, 2c, 3c and 4c show the integrated binding isotherms as a function of the molar ratio of drugs. The integrated binding isotherm of antibiotics-ivermectin binding is represented in figs. 2c, 3c or 4c (green rectangles in figs. 2b, 3b or 4b) is obtained by subtraction of integrated background heat of antibiotics-methanol titration (blue triangles in figs. 2b, 3b or 4b) from the integrated

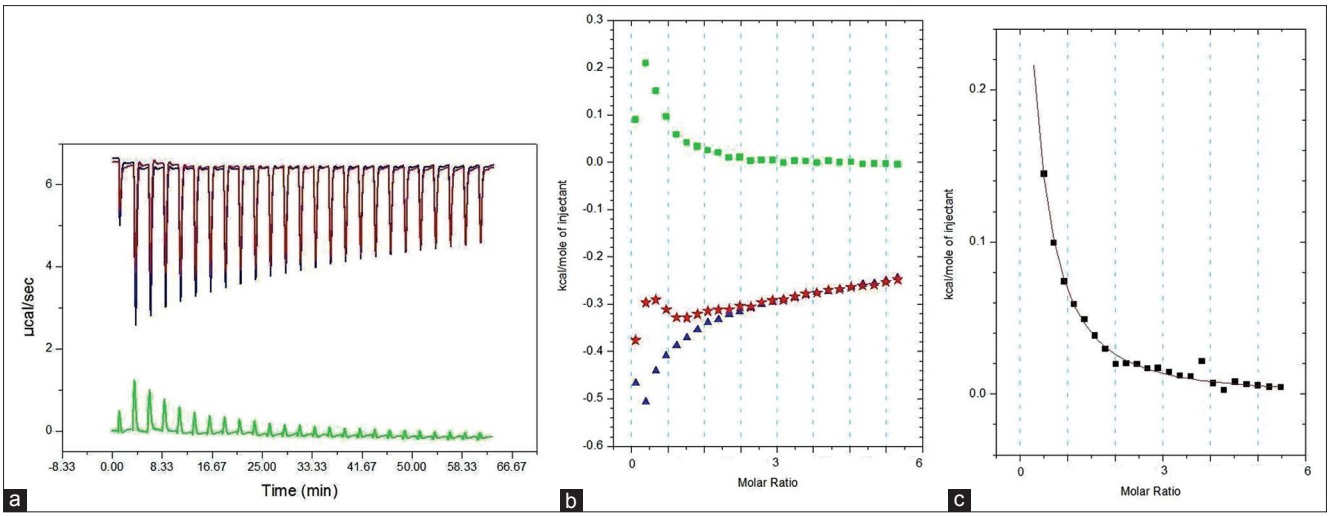

Fig. 4: ITC profiles of the binding of tetracycline HCl with ivermectin.
(a) The raw data heat changes produced by successive injections of tetracycline HCl into ivermectin solution. The raw data of tetracycline HCl-ivermectin titration is represented in red deflection peaks. The raw data of background heat changes are represented in blue peaks. The raw data of background heat changes were subtracted from that tetracycline HCl-ivermectin titration to yield the heat changes of tetracycline HCl-ivermectin binding (green peaks). (b) The binding isotherm of tetracycline HCl-ivermectin binding is represented in red stars, background heat in blue triangles and the integrated binding isotherm of tetracycline HCl-ivermectin binding (after subtraction of background heat) in green rectangles. (c) The integrated binding isotherm of tetracycline HCl-ivermectin interaction

TABLE 1: THE THERMODYNAMIC CONSTANTS OBTAINED BY ISOTHERMAL TITRATION CALORIMETRY

Substrate	N	K_a (1/M×10³)	ΔH (kcal/mol)	ΔG (kcal/mol)	ΔS (cal/K mol)
Ivermectin-ampicillin	3.6±1	1.9±0.4	−0.575±0.05	−4.55	17±1
Ivermectin-chloramphenicol	1.2±1.4	10±0.06	0.019±0.001	−5.6	18±1.3
Ivermectin-tetracycline HCl	ND	ND	ND	ND	ND

The thermodynamic constants obtained by isothermal titration calorimetry for the association of ivermectin with chloramphenicol, ampicillin and tetracycline HCl. The one set of sites binding model is used for fitting the data

heat changes of antibiotics-ivermectin binding (red stars in figs. 2b, 3b or 4b).

From the data of the given figures, we can determine the stoichiometry, binding affinity, the binding enthalpy and entropy. The stoichiometry (the number of antibiotic molecules that can bind to one molecule of ivermectin) is obtained at half saturation value. The binding affinity equals to the slope of the binding curve. The mechanism of antibiotic-ivermectin binding can be concluded by analysing the enthalpy and entropy of binding. The best fit of data was obtained by one set sites model. We tried several other fitting models, e.g., two sets sites model and sequential binding model; however, we obtained values with markedly high errors.

The isothermal titration experiments allowed for determination of the binding affinity between the drugs. Results indicated moderate binding affinity between ivermectin with ampicillin and tetracycline HCl (Table 1). The binding of chloramphenicol with ivermectin ($K_a^{chloramphenicol}$)=10,000 1/M, while the binding of ampicillin with ivermectin ($K_a^{ampicillin}$)= 1900 1/M. Thus, chloramphenicol binds ivermectin 5-folds stronger than ampicillin. By analysing the isotherms of tetracycline HCl binding, we were unable to detect the binding parameters with certainty. Exothermic signal was obtained, indicating a certain degree of binding can occur. However, the stoichiometry of binding was very low, from which there was error in estimating the binding affinity and other parameters.

Results indicated that ivermectin binds with the test antibiotics with different stoichiometry. The stoichiometry of ampicillin-ivermectin titration and chloramphenicol-ivermectin titration was 3 and 1.2, respectively. Thus, about three molecules of ampicillin can bind to one molecule of ivermectin and one molecule of chloramphenicol with one molecule of ivermectin. However, no binding stoichiometry can be detected with tetracycline HCl-ivermectin titration. Close inspection of ivermectin-tetracycline titration curve reveals sigmoidal curve, that is binding process may take place. However, the binding constant as well as the thermodynamic parameters was with high standard error.

An endothermic process accompanies the binding of chloramphenicol with ivermectin (fig. 3c). The enthalpic value of chloramphenicol-ivermectin binding (ΔH) was +0.019 kcal/mol. The positive sign indicates unfavourable enthalpic conditions, which indicates the lack of electrostatic attraction between chloramphenicol and ivermectin, lack of bond formation and bringing chemically repulsive groups near each other. The value of ΔS was 18 cal/K mol, that is, reflecting the change of ivermectin conformation to adapt the binding. In conclusion, the binding of chloramphenicol with ivermectin is accompanied by entropic changes and change in conformation of ivermectin. In contrast, exothermic process accompany the binding of ampicillin with ivermectin that is active interaction is present between the two molecules including exchange of hydrogen atoms and bonding. The positive ΔS is also an indicative for changes in ivermectin conformation.

The presence of other drugs was found to affect the bioavailability of ivermectin. For instance, the serum levels of ivermectin were affected if given with danoflxacin, dexamethasone and florfenicol[15-17]. To the best of our knowledge, there is no available data regarding the change in pharmacokinetics or drug bioavailability of ivermectin, if given in combination with chloramphenicol or ampicillin. Furthermore, the binding properties and interaction of these drugs is not previously studied. In general, the binding interaction between two or more of chemotherapeutics is not well understood.

In this report, we provide evidence that there is a binding interaction between ivermectin and two commonly used antibiotics, chloramphenicol and ampicillin. This result is considered as the first study of this type of interaction and is considered as a primer for further studies of ivermectin and other antibiotics interactions.

REFERENCES

1. Campbell WC, Fisher MH, Stapley EO, Albers-Schönberg G, Jacob TA. Ivermectin: A potent new antiparasitic agent. Science 1983;221:823-8.
2. Yacob HT, Mistre Ch, Adem AH, Basu AK. Parasitological and clinical responses of lambs experimentally infected with Haemonchus contortus (L3) with and without ivermectin treatment. Vet Parasitol 2009;166:119-23.
3. Dryden MW, Payne PA. Preventing parasites in cats. Vet Ther 2005;6:260-7.
4. Paterson TE, Halliwell RE, Fields PJ, Louw ML, Louw JP, Ball GS, *et al*. Treatment of canine-generalized demodicosis: A blind, randomized clinical trial comparing the efficacy of Advocate (Bayer Animal Health) with ivermectin. Vet Dermatol 2009;20:447-55.
5. Francisco I, Sánchez JA, Cortiñas FJ, Francisco R, Mochales E, Arias M, *et al*. Clinical trial of efficacy of ivermectin pour-on against gastrointestinal parasitic nematodes in silvopasturing horses. Equine Vet J 2009;41:713-5.
6. Gutman J, Emukah E, Okpala N, Okoro C, Obasi A, Miri ES, *et al*. Effects of annual mass treatment with ivermectin for onchocerciasis on the prevalence of intestinal helminths. Am J Trop Med Hyg 2010;83:534-41.
7. Pereyra-Rodríguez JJ, Bernabeu-Wittel J, Conejo-Mir MD, Ruiz-Pérez de Pipaón M, Conejo-Mir J. Treatment of cutaneous myiasis associated with scalp psoriasis in a 13-year-old girl with oral ivermectin. J Am Acad Dermatol 2010;63:908-9.
8. Kandeel M, Kitade Y. Binding dynamics and energetic insight into the molecular forces driving nucleotide binding by guanylate kinase. J Mol Recognit 2011;24:322-32.
9. Kandeel M, Kitade Y. Substrate specificity and nucleotides binding properties of NM23H2/nucleoside diphosphate kinase homolog from Plasmodium falciparum. J Bioenerg Biomembr 2010;42:361-9.
10. Kandeel M, Miyamoto T, Kitade Y. Bioinformatics, enzymologic properties, and comprehensive tracking of *Plasmodium falciparum* nucleoside diphosphate kinase. Biol Pharm Bull 2009;32:1321-7.
11. Kandeel M, Kitamura Y, Kitade Y. The exceptional properties of Plasmodium deoxyguanylate pathways as a potential area for metabolic and drug discovery studies. Nucleic Acids Symp Ser (Oxf) 2009;53:39-40.
12. Freire E. A thermodynamic approach to the affinity optimization of drug candidates. Chem Biol Drug Des 2009;74:468-72.
13. Zorko M, Jerala R. Alexidine and chlorhexidine bind to lipopolysaccharide and lipoteichoic acid and prevent cell activation by antibiotics. J Antimicrob Chemother 2008;62:730-7.
14. Kandeel M, Kitade Y. Analysis of the molecular interactions and complexation of chloroquine with bovine serum albumin. Drug Metabol Drug Interact 2011;27:41-6.
15. Areskog M, von Samson-Himmelstjerna G, Alvinerie M, Sutra JF, Höglund J. Dexamethasone treatment interferes with the pharmacokinetics of ivermectin in young cattle. Vet Parasitol 2012;190:482-8.
16. Ballent M, Lifschitz A, Virkel G, Sallovitz J, Maté L, Lanusse C. *In vivo* and *ex vivo* assessment of the interaction between ivermectin and danofloxacin in sheep. Vet J 2012;192:422-7.
17. Atef M, El-Gendi AY, Amer AM, Abd El-Aty AM. Effect of three anthelmentics on disposition kinetics of florfenicol in goats. Food Chem Toxicol 2010;48:3340-4.

Accepted 22 December 2012
Revised 11 December 2012
Received 25 October 2011
Indian J. Pharm. Sci., 2012, 74 (6): 592-596