J. Mater. Environ. Sci. 5 (1) (2014) 207-214
ISSN : 2028-2508
CODEN: JMESCN

Antimicrobial Activity of Naturally occurring Antibiotics Monensin, Lasalocid and their Metal Complexes

M. Mimouni[1,*], F.Z. Khardli[1], I. Warad[2], M. Ahmad[3,4], M.S. Mubarak[5,*], S. Sultana[3], T.B. Hadda[1,*]

[1]*Laboratoire de Chimie des Matériaux, Faculté des Sciences, Université Mohammed Premier, 60000 Oujda, Morocco*
[2]*Department of Chemistry, AN-najah National University, Nablus, Palestine.*
[3]*School of Chemical Engineering Universiti Sains Malaysia, 14300, Nibong Tebal, Penang, Malaysia.*
[4]*Neutraceutical Laboratory, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan – 45320*
[5]*Department of Chemistry, Faculty of Science, The University of Jordan, Amman 11942, Jordan*

Received 9 July 2013, Revised 15 July 2013, Accepted 16 July 2013,
**Correspondence to: E-mail: taibi.ben.hadda@gmail.com (T.B. Hadda), mimouni_8@yahoo.ca (M. Mimouni)*

Abstract
This paper focuses on evaluation of *in vitro* antimicrobial activity of two natural antibiotics, monensin (**1**) and lasalocid (**2**) and their metal complexes with Tl(I) (**3** and **4**), La(III) (**5**) and Gd(III) (**6**) against *Mycobacterium tuberculosis*, *Saccharomyces cerevisiae* and *Fusarium oxysporum f.sp albedinis* using POM analyses. Results indicated that monensin (**1**) and its Tl(I) complex (**3**) show remarkable bioactivity with MIC values of 0.15-0.34 µM and 0.1-0.23 µM against *Fusarium oxysporum f.sp albedinis* and *Saccharomyces cerevisiae* yeast, respectively. Preliminary screening results of selected ionophores exhibited potent antimicrobial activity; these ionophores could be considered as good potential drug candidates as antifungal agents against various fusarioses and also as potential antitubercular agents for future discovery of new drug design.

Keywords: Lasalocid; Monensin; Antifungal; Anti-tubercular; Ionophore, Tl(I), Gd(III) and La(III) complexes, Petra/Osiris/Molinspiration (POM) Analyses.

1. Introduction

Lasalocid and Monensin belong to the family of the polycyclic carboxylic polyethers; they are well known natural antibiotics isolated mainly from fungal mycelium of *Streptomyces cinnamonensis* (Actinomycetes) [1, 2]. From a bioinorganic chemistry point of view, these compounds are endowed with some unique properties such as the ability to form stable complexes with monovalent, divalent and trivalent cations and to facilitate the passage of metal cations across lipid membranes of cells through antiport mechanism [3]. In the literature, among the various ionophores reviewed by Pressman and Fahim [4], only three were known to have reliable broad commercial uses while the most important of which are monensin and lasalocid (Figure 1).

In the biological environment, these natural antibiotics work as inhibitors of the cell growth and are known due to their characteristic influence on Golgi apparatus through cellular secretary processes. The possibility of using such ionophores as biological probes was well documented [5]. However, it was only after the discovery of monensin and its extensive use in the beef and dairy industries to prevent coccidiosis [6] that people realized the economic importance of this class of antibiotic.

On the other hand, the effect of this antibiotics group on filamentous mushrooms, which are pathogenic filamentous fungi (molds), has been less thoroughly studied than the effect on yeasts. Indeed, the species *Fusarium oxysporum* is a group of mushrooms which commonly occur as fungal organizations in cultivated fields; they constitutes about 40-70% of the total flora fusarienne. This species is known by its diverse morphological and physiological behaviour either as saprophytic or parasitic life forms on other plants. According to an estimate about 80 forms of *Fusarium oxysporum* were identified as pathogenic. Among these, some special forms are known to induce a number of diseases in other plants such as *Fusarium oxysporum f.sp apii* which attacks celery and pea crops, *Fusarium oxysporum f.sp vasinfectum* is pathogenic to cotton, tobacco and the alfalfa plants. While *Fusarium oxysporum f.sp cubens* is known as causal agent of the fusariose in

banana tree and *Fusarium oxysporum f.sp albedinis* act as causal agent for vascular fusariose in date palm (Bayoud), these two most serious diseases are recently reported in Morocco.

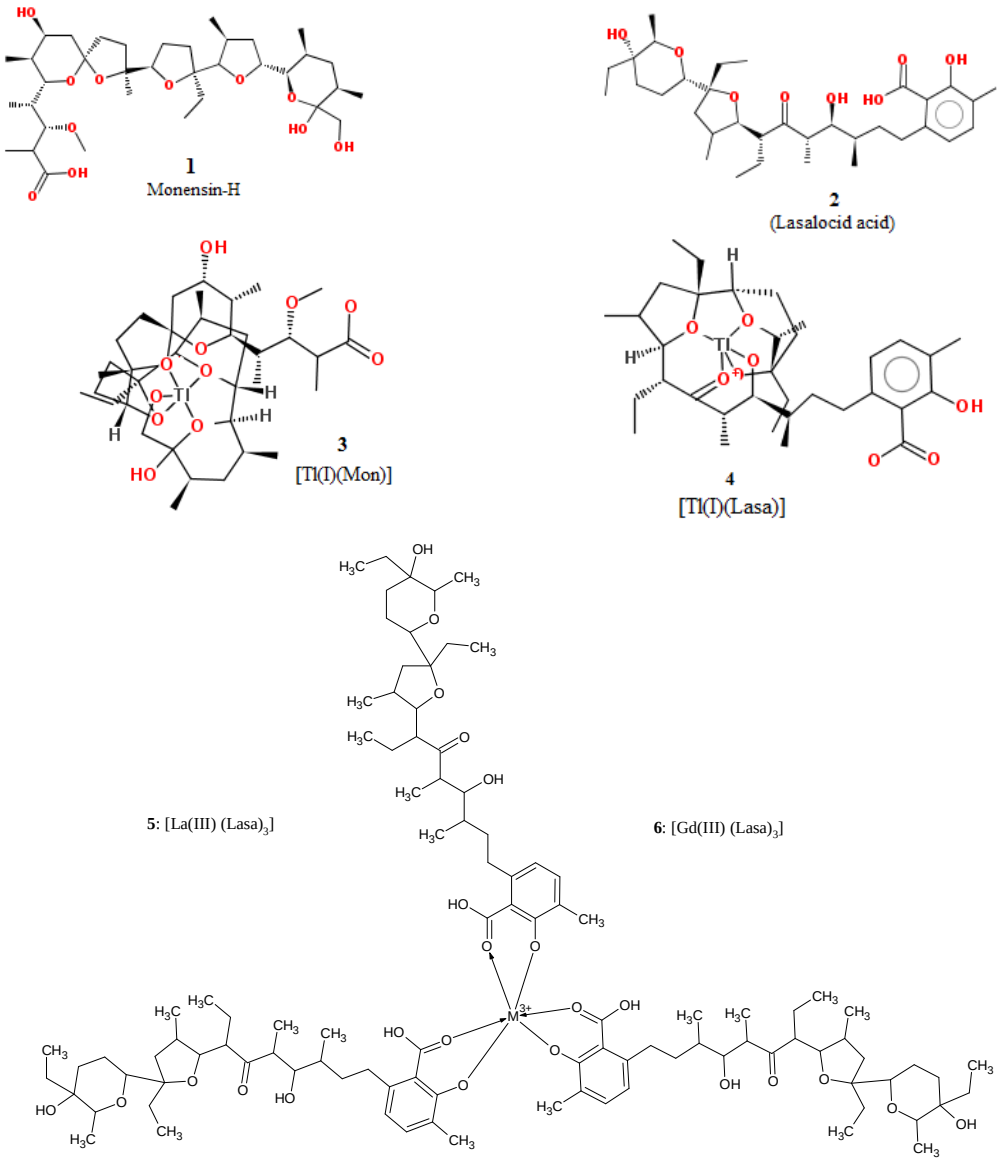

Figure 1. Structures of natural antibiotics: monensin acid (**1**), lasalocid acid (**2**) and their Thalium(I), Lanthanide(III) and Gadolinium(III) complexes **3-6**.

The need for novel and more effective antifungal drugs development is an absolute necessity and remains a very important area of research in an effort against pathogens, especially those which have devastating effects both on plants and human. In view of the wide interest in the search for effective and more versatile antifungal natural origin based drugs, we describe herein the synthesis of a number of metal complexes of the commercially available ionophores, monensin (**1**) and lasalocid (**2**); this is based on the strategy to couple the properties of the ligand with and those of the cations, lanthanides and the thallium, which are strong Lewis acids and have a high number of coordination. The antifungal activity of the antibiotics **1** and **2** and their metal complexes against *Fusarium oxysporum f.sp albedinis* and a yeast stock *Saccharomyces cerevisiae* cultured on solid media was evaluated; the aim is to have new leads in the development of new drugs design in forthcoming investigations for wider acceptance and circulation as protective measures. This will hopefully act as milestone study with significant impacts of complexation on the biological activities.

2. Experimental protocols

2.1. Micro-organisms and conditions of cultures

The antifungal activity of the prepared compounds was evaluated against *Fusarium oxysporum* f.sp. *albedinis*. A stock culture of *Fusarium* was isolated from a date palm infected by the vascular fusariose. Organism were grown aerobically in liquid Czapek medium; 2 g/L of $NaNO_3$, 1 g/L of K_2HPO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$), 0.01 g/L of $FeSO_4 \cdot 7H_2O$ and 30 g saccharose/L. The pH was adjusted to 5.4 with citric acid. All liquid cultures were inoculated into 100 mL Erlenmeyer flasks with narrow collars containing 20 mL of medium at 25 °C, in a water bath, with horizontal rotary agitation at 70 rpm. The growth was followed by measuring the optical density with a Cecil spectrophotometer at a wavelength of 620 nm and by counting the organisms on a cell of Malassez under an optical microscope. Cultures on solid medium were seeded in 50 mm in diameter Petri plat in a thermostatically controlled drying oven at 28 °C. The growth was followed by counting colonies of yeast and bacteria and by measuring the diameter of the *Fusarium* mycelium.

2.2. Antibacterial bioassay (in vitro)

Anti-tuberculosis activity assays were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) at the Southern Research Institute, Birmingham, AL, USA. Screening was conducted at 6.25 µg/mL against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) using the Microplate Alamar Blue Assay (MABA) [7].

3. Chemistry

3.1. Synthesis and characterisations of ionophores (1-2) and their complexes (3-6)

The ionophores in acid (AH) and anion (A^-) forms were prepared from the commercially available sodium salt (Na-A) (Chem Sigma. Corp), and were then purified according to the method described by Juillard and co-workers [8]. On the other hand, the thallium salt (Tl-A) of monensin was prepared according to a procedure outlined by Juillard and his research team [9]. Similar procedures were employed for the synthesis of lasalocid. Salts of lasalocid lanthanum (La-A_3) and gadolinium (Gd-A_3) were prepared and purified according published procedures [10]. NMR spectral data were acquired on a Brücker MSL 300 and Brücker AC 400 spectrometers. Details of the methods employed for various experiments are described elsewhere [11]. For each compound, 1D (^1H, ^{13}C and J-MOD) and 2D homo-nuclear and hetero-nuclear experiments (COSY and ^{13}C-^1H) NMR experiments were performed; these experiments showed correlations that helped in the ^1H- and ^{13}C-signal assignments to the different carbons and their attached, and/or neighboring hydrogen. ^1H and ^{13}C chemical shifts were given and certain constants of proton-proton coupling were calculated. Carbone–thallium couplings for the two ionophore thallium complexes, **3** and **4** were detected.

4. Pharmacology

4.1. Antimicrobial activity of metal-ionophores (in vitro)

Our results indicated that these micro-organisms, *Mycobacterium tuberculosis*, *Saccharomyces cerevisiae* and *Fusarium oxysporum* f.sp *albedinis*, were found to be more sensitive to monensin and its metal complexes than to lasalocid and its metal complexes analogues. Monensin and lasalocid are molecules with chains which can pass from the quasi-open form to another quasi-closed form stabilized by hydrogen bonds between carboxylate, and by ketone and hydroxyl groups. The external surface of the complex ion-ionophore consists of alkyls groups, which increase its solubility in nonpolar solvents. In biological systems, these complexes are freely soluble in the double-layered phospholipid, one of the cellular membranes, and diffuse easily through this membrane from one interface to another. This disturbs the Na^+/H^+ exchanges and the normal operation of the cell [12]; consequently, the quantity of transferred ions can be very high [13]. This transfer depends on several factors such as the concentration of K^+ ions in the external medium, the type and concentration of the perméant ion which accompanies K^+ and of the physicochemical properties of the plasmic membrane. Our findings revealed that complexes of very high molecular masses such as lasalocid-lanthanum and lasalocid-gadolinium were found to be most effective against two tested mushrooms. It could be explained by the fact that the number of ligands (three ligands) is an important factor, if it is more, the complex is hydrophobic, and therefore, its passage through the membrane barrier is easy and suitable. In contrast, this was not the case with monensin because it is a more rigid molecule than lasalocid and as a result, it has less labiality to form hydrophobic complexes. Furthermore, it has been observed that processes on the membrane by action of these ionophores generate a cascade of disturbances at the cellular level. As an example, Poli et al. [14] showed that monensin

induced at *Candida albicans* (a pathogenic yeast) an inhibition of germination, as well as modifications of the morphogenesis due to the accumulation of chitin involving thickening at the parietal level. In the case of filamentous mushrooms, Podila and collaborators [15] reported that the ionophore inhibits the secretion of the cutinases at *Fusarium solani* and *Colletotrichum gloeosp-orioides*.

4.2. Biological evaluation of compounds 1-6

Various species of lasalocid and its complexes, exhibited significant inhibition against the growth of the two mushrooms *Saccharomyces cerevisiae* and *Fusarium oxysporum f.sp albedinis*. The degree of this inhibition varies from one species to another. With *Fusarium* (Table 1), the complex which shows a greater inhibiting activity is lasalocid-lanthanum with an MIC value of 0.63 µM, followed by the lasalocid gadolinium complex, and the lasalocid-thallium. The free lasalocid acid was observed as the least effective among them. Moreover, the same phenomenon was observed with yeast (Table 2) with a sensitivity less pronounced than that of *Fusarium* with respect to this ionophore. Bioassay results with yeasts indicated that the lasalocid-lanthanum complex is approximately 12 times more active than the lasalocid acid itself. Similarly, the monensin acid appears approximately 2 times more effective than the monensin-thallium complex as shown in Tables 1 and 2. Comparison of biological activity of these complexes is illustrated in Table 3.

4.2.1. Determination of anti-fungal activity against Fusarium oxysporum strains

Fusarium oxysporum is a highly pleomorphic microorganism whose diverse forms can behave both as saprophytes as well as parasites in various plants, and among the latter, different degrees of virulence can arise. Some of them can infect plants belonging to different families, for example celery and peas. *F. oxysporum* f.sp *albedinis*, is responsible for the vascular fusariose of the date palm, is a pest that ranks with *F. oxysporum* f.sp *cubense* (causal agent of the fusariose of the banana tree) high on the list of serious agricultural problems. Antifungal activity was assessed against the *albedinis* strain. Monensin, lasalocid and their complexes were dissolved in dimethylsulfoxide with 50% ethanol, and then added at different concentrations into the solid culture media. The percentage inhibition of growth was expressed in terms of the ratio of diameter of the treated mycelium to that of the untreated control (Table 1).

Table 1. Antifungal activity of **3-6** complexes against *Fusarium oxysporum* f.sp *albedinis* (*f.o.s*).

Compd.	Formula	Percent growth Inhibition (C in µg/mL)			Assessment
		C_1 = 1.5	C_2 = 3	C_3 = 6	
1	Monensin-H	42.0	75	100	**Active**
2	Lasalocid-H	2.40	14	38	Inactive
3	[Tl(I)-(Mon)]	35	57	87	**Active**
4	[Tl(I)-(Lasa)]	0.00	6.7	17	Inactive
5	[La(III)-(Lasa)$_3$]	2.40	7.30	29	Inactive
6	[Gd(III)-(Lasa)$_3$]	0.00	3.30	23	Inactive

Results presented in Table 1 reveal that the most effective inhibitors are **1** and **3** with 100 and 87% inhibition, respectively, against the growth of *Fusarium* in the presence of 6 mg/L. On the other hand, molecules **4**, **5**, **6**, and **2** are less effective but show appreciable growth inhibition (17-38% Inhib) at comparable concentration (6 µg/L); these compounds inhibit the growth weakly (<50%) and thus are considered essentially inactive. In fact, these results are promising and merit further investigations.

Table 2. Antifungal activity of compounds **3-6** against *Saccharomyces cerevisiae (Sac. Cer.).*

Compd.	Formula	Percent growth Inhibition (C in µg/mL)			Assessment
		C_1 = 1.5	C_2 = 3	C_3 = 6	
1	Monensin-H	75	100	100	**Active**
2	Lasalocid-H	0.0	0.0	90	**Active**
3	[Tl(I)-(Mon)]	65	90	100	**Active**
4	[Tl(I)-(Lasa)]	0.0	30	50	Inactive
5	[La(III)-(Lasa)$_3$]	10	20	90	**Active**
6	[Gd(III)-(Lasa)$_3$]	0.0	10	70	**Active**

Molecules **1** and **3**, with the best activity contain a monensin ligand on each of the functionalised chain (CH_2-OH). Replacement of the (HO-C-CH_2-OH) group of **1** by (H_3CH-C-OH) in compound **2** results in a drastic loss of antifungal activity. The loss of activity could be attributed to the loss of the pharmacophore site, rigid character that the spiranic carbon confers on molecule **1**. Hydrophobic molecules with rigid and planar structures, such as aromatic or heterocyclic rings, have been shown to have the ability to penetrate into membranes and induce localized permeability changes leading to leakage out of the membrane.

Table 3. MIC of antifungal activity of compounds **1-6**.

Compd.	M. W. (g/mole)	Bayoud Assay		Sac. Cer. Assay	
		MIC (µg/mL)	MIC (µM)	MIC (µg/mL)	MIC (µM)
1	671	0.11	0.15	0.07	0.10
2	591	1.31	2.20	3.50	5.93
3	874	0.31	0.34	0.21	0.23
4	794	1.50	1.90	2.01	2.52
5	1908	1.21	0.63	1.01	0.52
6	1926	2.01	1.04	2.51	1.31

4.2.2. Evaluation of anti-tubercular activity (in vitro)

Compounds **1-6** have been evaluated as anti-tuberculosis agents through the TAACF tuberculosis screening program and findings are given in Table 4. As indicated in Table 4, compounds **3** and **1** have shown significant potency to inhibit the growth of *Mycobacterium tuberculosis* H_{37}Rv using the Alamar assay at the first level adopted for *in vitro* screening. Compounds **2, 4**, and **5** on the other hand, displayed modest *in vitro* activity (less than 30%). Not surprisingly, the two compounds **3** and **1** of these tested natural derivatives are currently classified as the best anti-tubercular candidates and will be examined at the *in vivo* stage of the tuberculosis screening program at Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) of United States of America.

Table 4. Anti-tubercular activity of compounds **1-6**.

Compd.	TAACF code	Assay	MIC (µg/mL)	Inhibition%	Activity
1	155602	Alamar	<6.25	99	**positive**
2	155603	Alamar	>6.25	25	negative
3	155598	Alamar	<6.25	97	**positive**
4	155599	Alamar	>6.25	17	negative
5	155600	Alamar	>6.25	14	negative
6	155601	Alamar	>6.25	5	negative

The monensin derivatives, **3** and **1** are the best active substances to have been evaluated as anti-tubercular agents in our laboratories. Accordingly, an effort was initiated to establish a pharmacophore hypothesis to delineate the requirements of the active site *via* a comprehensive program of analogue synthesis and evaluation of the effects of structural modification(s) on anti-tubercular activity of **2**. The chosen strategy was formulated by changing the physico-chemical properties of the parent structure by complexing **2** to various metals (Tl, La and Gd). The resultant *in vitro* and *in vivo* effects of metal centre alterations in sphere coordination will be examined for these complexes. Neither the free ligand nor the thalium, lanthanide or gadolinium complexes **4-6** exhibited anti-antitubercular activity (%Inhib<50%). The modulating anti-tubercular effect(s) of substituents having different pharmacological affinity, located at the tetrahydrofuryl site comprising the A-ring of **1**, were ascertained next. A combination of hydroxyl and hydroxymethyl substituents located, respectively, at the α,β positions on the furyl ring of **1** generated the higher anti-tubercular activity relative to lasalocid (**2**) and its complexes **4-6** in all of the bacteria and yeast lines tested. However, in general coordination of lasalocid as ligand to Tl, La or Gd proved to be unhelpful.

To sum up, we postulate that the absence of the (HO-CH_2-C-OH) pharmacophore site and the strong tendency to form species with weak solubility (MIC > 6.25 µg/mL) in the predominant form is likely to be responsible for the lack of biological activity observed with these natural lasalocid complexes. This hypothesis

seems to be correct; by changing lasalocid with monensin we have been able to modulate the degree of interaction of the compound with *Mycobacterium Tuberculosis* H37Rv bacteria, *Saccharomyces cerevisiae* and *Fusarium oxysporum* f.sp *Albedinis* yeasts.

5. POM analyses of compounds 1-6

5.1. Osiris calculations

With our recent publications on drug design combination of various pharmacophore sites [16-20], it would be possible to predict the type of bioactivity of candidate drugs. This is achieved by using a combined electronic/structure docking procedure as illustrated in Table 5. The remarkably well behaved mutagenicity of divers' synthetic molecules classified in data base of CELERON Company of Switzerland can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA.

Data presented in Table 5 indicate that, all structures are supposed to be non-mutagenic when run through the mutagenicity assessment system and as far as irritating and reproductive effects are concerned, all compounds are at low risk. The log P value of a compound, which is the logarithm of its partition coefficient between *n*-octanol and water, is a well-established measure of the compound's hydrophilicity. Low hydrophilicity and therefore, high log P values may cause poor absorption or permeation. It has been shown that **1** has a reasonable probability of being well absorbed; its log P value must not be greater than 5.0. Based on this argument, compounds **1–6** having clogP values under the acceptable criteria should be active. The geometrical parameter and the aqueous solubility of a compound significantly affect its absorption, distribution characteristics, and bioactivity. Typically, a low solubility goes along with a bad absorption and therefore, the general aim is to avoid poorly soluble compounds. Data pertaining to antibiotics **1**, **2** and their complexes **3-6** are reported in Table 5.

Furthermore, we have calculated overall drug-score (DS) for the complexes **3–6** and compared the values with those of standard drugs **1** and **2** as shown in Tables 5 and 6. The DS combines drug-likeness, CLog*P*, log*S*, molecular weight, and toxicity risks in one handy value that may be used to judge the compound's overall potential to qualify for a drug. The reported standard compounds **1** and **2** indicate that both compounds have good DS, while the DS values for the rest of the series (compounds **3-6)** were not possible to evaluate by using Osiris software. This indicates that some supplementary parameters in drug design should be taken into consideration in Osiris conception.

Table 5. Osiris calculations of toxicity risks and drug-score of compounds **1-6**.

Compd.	Toxicity risks				Drug-score			
	MUT	TUMO	IRRI	REP	CLP	S	DL	DS
1					3.14	-5.39	2.21	0.35
2					5.77	-6.12	2.67	0.25
3	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND

: not toxic ; : slightly toxic; : highly toxic. ND: Not determined by Osiris program.
[a] MUT: mutagenic; TUMO: tumorigenic; IRRI: irritant; REP: reproductive effective. [b] CLP: cLogP, S: Solubility, DL: Druglikness.

This fundamental limitation of Osiris in computational chemistry led us to explore the Drug likeness obtained by using a more performant software, Molinspiration, a program of docking and virtual screening of drugs.

5.2. Molinspiration calculations

The method is very robust and is able to process practically all organic and most organometallic molecules. Molecular Polar Surface Area TPSA is calculated based on the methodology published by Ertl et al [26 21]

which is virtually a sum of fragment contributions; O– and N– centred polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability and blood–brain barrier penetration [22]. Prediction results of compounds **1–6** molecular properties (TPSA, GPCR ligand and ICM) are given in Table 6.

Table 6. Molinspiration calculations of compounds **1-6**.

Compd.	Physico-chemical properties [a]					Drug likeness [b]				
	TPSA	O/NH	VIOL	ROTB	VOL	ICM	KI	NRL	PI	EI
1	153	4	2	10	648	-0.75	-0.50	-0.29	0.11	0.13
2	134	4	2	13	585	-0.52	-0.49	0.17	0.09	0.16
3	127	3	2	6	661	-1.06	-0.80	-0.58	-0.03	-0.56
4	98	2	1	8	596	-0.46	-0.34	0.01	0.05	-0.11
5	350	9	4	39	1756	-6.36	-6.50	-6.33	-6.04	-6.12
6	350	9	4	39	1756	-6.36	-6.50	-6.33	-6.04	-6.12

[a] TPSA: Total polar surface area, O/NH: O---HN interraction, VIOL: number of violation , VOL: volume;
[b] ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand. PI: Protease inhibitor; EI: Enzyme inhibitor.

In contrast to low ability of series **1-6** to act as Kinase inhibitors (KI is negative: from -6.50 to -0.34), they present various potentials as protease inhibitors (0.09, 0.11 and 0.05 for **1**, **2** and **4** respectively) and as enzyme inhibitors (0.13 and 0.13 for **1** and **2,** respectively).

Conclusion

A number of important points emerge concerning the biological properties of monensin (**1**), lasalocid (**2**) and their metal complexes and their interaction with bacteria and yeasts. The remarkable bioactivity results against different pathogens were achieved using monensin and its organometalic derivatives, while encouraging for purposes of new drug design, confirm that most of these compounds could very likely be used without great risk of toxicity in diverse pharmaceutical applications [22-25]. Based on such promising biological properties of these naturally occurring compounds, these can be useful as potential anti-tubercular agents leads to discovery and synthesis of novel antifungal drugs. Monensin (**1**) and its complex **3,** which can evidently recognise specific biological sites, could have a potential for gene targeting which merits investigation based on its capacity to inhibit access of activators or repressors to regulatory sites in DNA controlling gene expression. These results are encouraging since it was shown that these compounds do not practically disturb the mechanisms of the protein synthesis and do not act on the quantities of ATP present in the cell [26]. Further investigations to highlight these evidences will be reported in the near future.

Acknowledgements - We are indebted to both Prof. G. Geminet and Prof. J. Balzani for providing their help in taking the NMR and mass spectra and in carrying out the bioassays. Prof. T. Ben Hadda is thankful to TAACF/USA for anti-tubercular screening.

References

1. Berger J., Rachlin A.I., Scott W.E., Sternbach L.H., Goldberg N.W., *J. Am. Chem. Soc.* 73 (1951) 5295.
2. Hankey M.E., Hoen M.H., *Antimicrob. Agents Chemother.* (1967) 349.
3. Mimouni M., Perrier S. , Pointud Y., Juillard J., *J. Sol. Chem.* 22 (1993) 769.
4. Pressman B.C., Fahim M., *Ann. Rev. Pharmacol. Toxicol.* 22 (1982) 465.
5. Reed P.W., Bokoch G.M., *Polyether antibiotics Naturally Occurring acid ionophores*, 1 (1982) 369.
6. (a)- Westley J.W., *Polyether antibiotics- Naturally Occurring acid ionophores*. 1 (1982) 1-20. (b)- Munir K., Muneer M.A., Tiwari A., Chaudhry R.M. and Muruganandan S., *Vet. Res. Commun.*, 31 (2007) 909.
7. Collins L., Franzblau S.G., Antimicrob. *Agents Chemother*. 41 (1997) 1004-1009.

8. Hébrant M., Pointud Y., Juillard J., *J. Phys. Chem.* 95 (1991) 3653.
9. Mimouni M., Hébrant M., Dauphin G., Juillard J., *J. Chem. Res. (M)* (1996) 278.
10. Mimouni M., Thèse de Doctorat d'Etat, Es-sciences, Université Mohamed Ier, (1998) N° 213/98.
11. Lyazghi R., Cuer A., Dauphin G., Juillard J., *J. Chem. Soc. Perkin. Trans.* 2 (1992) 35.
12. Sutko J.L., Besch H.R., Bailey J.C., Zimmerman G., Watanabe A.M., J. *Pharmacol. Exp. Ther.* 203 (1977) 685.
13. Pressman B.C., Annu, *Rev. Biochem*., 45 (1976) 501.
14. Poli F., Pancali S., Vannini G.L., *Eur. J. Cell. Biol.* 42 (1986) 79.
15. Podila G.K., Rosen E., San Francisco M.J.D. and Kolattukudy P.E., *Phytopathology*, 85 (1995) 238.
16. Ozcelik B., Sheikh J., Orhan I., Juneja H., Bennani B., Kerbal A., Cavalcanti S.M.T., Leite A.C.L., Hadda T.B., *Res. Chem. Intermediates* (2012).
17. Parvez A., Jyotsna M., Youssoufi M.H., Hadda T.B., *Phosphorus Sulfur*, 185 (2010) 1500.
18. Parvez A., Meshram J., Tiwari V., Sheikh J., Dongre R., Youssoufi M.H., Hadda T.B., *Eur. J. Med. Chem*. 45 (2010) 4370.
19. Jarrahpour A., Fathi J., Mimouni M., Hadda T.B., Sheikh J., Chohan Z.H., Parvez A., *Med. Chem. Res.* 21 (2012) 1984.
20. Jarrahpour A., Motamedifar M., Zareil M., Youssoufi M.H., Mimouni M., Chohan Z.H., Hadda T.B., *Phosphorus Sulfur*, 185 (2010) 491.
21. Ertl P., Rohde B., Selzer P., *J. Med. Chem.* 43 (2000) 3714.
22. Hebrant M., Mimouni M., Tissier M., Pointud Y., Juillard J., Dauphni G., *New J. Chem.*, 16 (1992) 999.
23. McGrego E.B., Solomon K.R., Hanson M.L., Arch. Environ. Contam. *Toxicol.* 53 (2007) 541.
24. Mc-Garvey J.A., Hamilton S.W., DePeters E.J., Mitloehner F.M., *Appl. Microbiol. Biotechnol*. 2009, DOI 10.1007/s00253-009-2229-8.
25. Mohebbi-Fani M., Nazifi S., Shekarforoush S.S., Rahimi M., *Vet. Res. Commun.* 30 (2006) 7.
26. Ledger P.W., Tanzer M.L., *Trends Biochem, Sci.* 9 (1984) 313.