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Azole-based compounds as antiamoebic agents: A perspective using theoretical calculations

Md. Mushtaque[a*], Shahzaib Ahamad[b,c], Meriyam Jahan[a], Kakul Hussain[d], Mohd. Shahid Khan[e]

[a*]*School of Physical and Molecular Sciences (Chemistry), Al-Falah University, Dhauj, Faridabad, Haryana, India.*

[b]*Department of Biotechnology, College of Engineering & Technology, IFTM, Lodhipur-Rajput, Delhi Road, Moradabad.*

[c]*Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi, India.*

[d]*Medical Lab Science Department, College of Applied Medical Sciences, Salmanbin Abdulaziz University, Wadi Adadawasir, Saudi Arabia.*

[e]*Department of Physics, Jamia Millia Islamia, New Delhi, India.*

Abstract

Diseases caused by protozoal organisms are responsible for significant mortality and morbidity worldwide. Amoebiasis caused by *Entamoeba histolytica* is an example of such diseases. In the quest of safe and effective antiamoebic agent, several heterocyclic moieties have been reported, out of which members of azole family (dioxazole, pyrazoline, tetrazole, triazole and thiazolidinone derivatives) attracted wide attention. This class of heterocyclic compounds emerged as a potential chemotherapeutic agent exhibiting promising antiamoebic activity with non-cytotoxic nature. In the present article, some important breakthroughs in this area have been discussed. To get an insight at supra-molecular level, computational studies like Lipinski's and DFT studies were carried out. Potent activity, chemical potential and hardness of active compounds based on theoretical calculation were explained. DFT study indicated that the LUMO energy level should lie between -1.34 to -0.54 eV to show high activity. We also observed that the LUMO level was mainly distributed over 2-methyl 5-nitro imidazole ring in most of the active compounds.

Keywords: Azo based Compounds, Antiamoebic activity, Lipinski's rule, Structure Optimization.

*****Corresponding author:* *E-mail:* mush.chem@gmail.com;mushtaquem81@ymail.com
Contact no: +91-9891717992

Table of content

1. Introduction

Protozoans, a eukaryotic unicellular organism, have the capability to destroy a multicellular organism by causing infectious diseases. Several ailments like malaria, giardiasis, amoebiasis, chagas, sleeping sickness and amoebic dysentery etc. have been associated with this class of organism, which affected a large number of populations. Amongst all, malaria and amoebiasis are the most common ones, which can be seen in all parts of world, especially in developing and underdeveloped countries.[1-4] Amoebiasis, caused by the species *Entamoeba histolytica* (*E. histolytica*) is the third most life-threatening disease after malaria and schistosomias.[5, 6] Although it is non-symptomatic in most of the cases, it infects over 50 million people per annum leading to 50,000 to 100,000 deaths annually[4]. Areas with high rates of amoebic infection incidence include India, Africa, Mexico, central and South America and Australia.[7-13] In addition to amoebic infections, they are also a potential reservoir for other bacteria's.[12]

The parasite exists in two forms: an infective cyst form (that can survive outside the body) and a motile pathogenic trophozoite form (that do not persist outside the body).[14, 15] The infective cyst form enters into the human body through food or water contaminated with fecal matters and liberate as trophozoites in the intestinal lumen. Then the trophozoites either invade and ulcerate the mucosa of the large intestine or simply feed on intestinal bacteria. However, *E. histolytica* remains in the colon as harmless commensally, but after a period of time it becomes devastating for human being causing dysentery, colitis, liver abscess, hemorrhagic colitis and extra intestinal abscess and amoebic brain abscess.[15, 16]

To curb this disease, drugs of both natural and synthetic origin (**Chart 1**) are being used.[17] In spite of excellent activity profile with high usage and demands of some common drugs like metronidazole (**1**), tinidazole (**2**), ornidazole (**3**), secnidazole (**4**), emetine (**5**), iodoquinol (**6**), diloxanidefuroate (**7**) and paromomycin (**8**) (**Chart 1**), side effects associated with these drugs compelled researchers to think for a better alternative.[17] In this context, several attempts have been made to synthesize/isolate new drug with same/better activity profile with no/less toxicity. Interestingly, some new synthetic azole-based molecules emerged as a potential candidate and are knocking the door to enter to the market (**Chart 2**).

Considering the importance of this class of molecule, we present here some recent advances made in the synthesis and biological activity of azole-based antiamoebic agents. Furthermore, a theoretical study was also carried out to make a rationale for the design of new drug. Since

computational studies play an important role in the drug discovery due to low cost, quick and acceptable results[18], the outcome of the present finding will be helpful for the researchers working in this area.

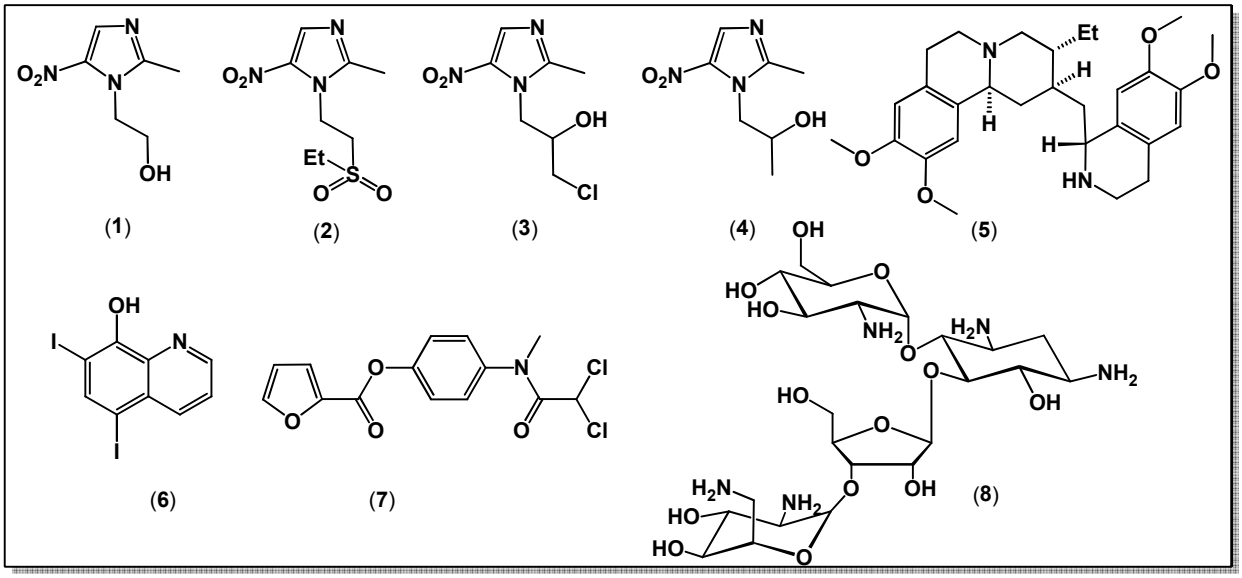


Chart 1: Structure of some commonly used antiamoebic agents.

2. Mechanism of antiamoebic drugs

For bacterial infections and pathogenic protozoan parasites, 2-methyl 5-nitro imidazole based drugs are being used for last five decades.[19, 20] Currently, 2-methyl 5-nitro imidazole derivatives in market are metronidazole (**1**), tinidazole (**2**), ornidazole (**3**) and secnidazole (**4**) and are highly recommended for the treatment of different stages of amoebiasis.[20, 21] Particularly metronidazole (**1**), tinidazole (**2**), ornidazole (**3**) are the main synthetic drugs.[21] The mechanism of action of 5-nitroimidazole derived drugs is based on the reduction of nitro group by nitro reductase enzyme like thioredoxin reductase (TrxR) or ferrodoxin.[22-25] The resulting nitro radical anion is a single-electron transfer reduction product[25], which further undergoes reduction to yield highly reactive nitroso species (**Fig. 1**). This species then binds to genetic materials/proteins/other bio-molecules to inhibit the activity of *E. histolytica*.[23, 25]

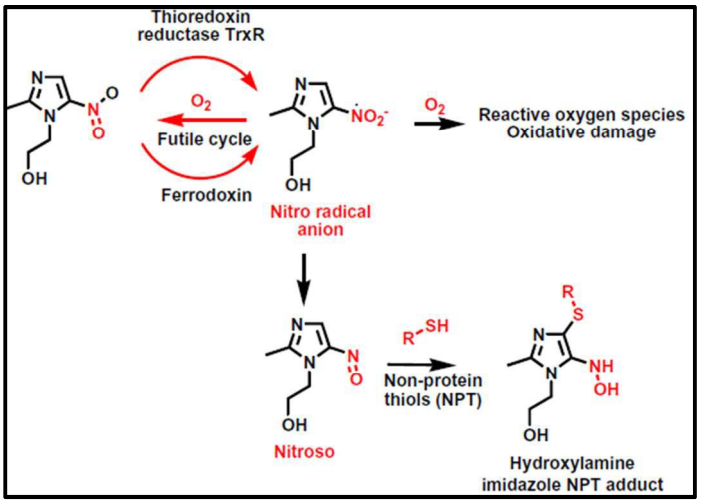


Fig. 1. Mechanistic pathway responsible for MNZ (**1**) activity.[26] Reprinted from Bioorganic & Medicinal Chemistry Letters, 22(17), A. Salahuddin, S. M. Agarwal, F. Avecilla and A. Azam, Metronidazole thiosalicylate conjugates: Synthesis, crystal structure, docking studies and antiamoebic activity, 5694-5699, Copyright (2012), with permission from Elsevier.

Similarly, tinidazole (**2**), a structural analogue of MNZ, is known to act by reducing itself to cytotoxic intermediates that covalently bind to DNA, causing irreversible damage.[27] Emetine (**5**) is an alkaloid originally extracted from *Ipecac* roots. It kills trophozoites mainly by inhibiting protein synthesis by blocking translocation of the peptidyl-tRNA from acceptor to donor site on the ribosome.[28-30]

3. Limitations of current antiamoebic drugs

During the last 50 years, numbers of compounds possessing amoebicidal activity have been isolated and synthesized.[17] Most of them are being in use either as single agent/in combination with antibiotics or other medications.[17, 28, 31] Chemically, most of the agents are derivatives of imidazoles, alkaloids, furan and quinolines. **Chart 1** represents some of the main drugs used for protozoal infection. The drugs used to treat amoebiasis are classified as tissue amoebicides and luminal amoebicides, depending upon the site of infection.[13, 32, 33] Metronidazole (**1**), tinidazole (**2**), ornidazole (**3**), secnidazole (**4**), emetine (**5**) and dehydroemetine are some of the tissue amoebicides, which kill amoeba in host tissue and organ.[34] Side-effects of MNZ (**1**) includes burning/numbness in foots or hands, confusion, dizziness, drowsiness, fever, nausea, headache, metallic taste, dry mouth, glossitis, urticaria, pruritus, urethral burning and dark colored urine etc.[35-38] Some studies have reported that this drug induces encephalopathy,[39, 40] shows genotoxicity and carcinogenicity too.[41, 42] Some recent reports have also demonstrated the *in-*

vitro generation of strains resistant to MNZ and other drugs.[28, 43] Tinidazole (**2**) shares same pharmacological profile and toxicity with MNZ like bitter taste, nausea, abdominal discomfort, anorexia, vomiting, and fatigueness. However, its toxicity persists for less time than MNZ.[27] Similarly, patients treated with secnidazole (**4**) were reported to feel nausea, gastralgia, change of taste, stomatitis, urticaria, rashes, leucopenia and others.[44] However, a detailed scientific study on human beings for secnidazole (**4**) side effects is not available, but this drug has been suggested as *category C* drugs, which means it may have adverse effect on the fetus.[45] Severe side effects of emetine (**5**) include cardiotoxicity, adrenergic (α_2) blocking activity, inhibition of dipeptidyl aminopeptidase IV etc.[30, 46, 47] To overcome these limitations, several new molecules were reported. However, new emetine derivatives / analogues were less toxic than the parent drug emetine itself, the biological activities of emetine derivatives / analogues were not so high so that it could be employed for clinical purposes. For a range of emetine derivatives / analogues, readers are suggested to read extensive reviews published on this topic.[17, 30, 46]

On the other hand, currently used luminal agents are ornidazole **(3)**, iodoquinol **(6)** and diloxanidefuroate **(7)** which are active only in intestinal lumen.[34] However, in majority of cases, nitro imidazole-based tissue amoebicide could effectively control the epidemic, but in some cases, it become necessary to administer nitro imidazole-based tissue amoebicide followed by paromomycin or the second line drug diloxanidefuroate **(7)** to take care of luminal infection.[48, 49] Similar to the tissue amoebicides, luminal amoebicides are also associated with toxicities and side effects. For example, the common side effect of paromomycin (**8**) is diarrhea, which causes trouble to both the patients and the physician. Overall, both classes of antiamoebic drugs are associated with one or more side effects including resistant development. These necessitate the development of novel drug with good therapeutic activity and less/no-risky side effects.

4. Heterocyclic azole based compounds with promising antiamoebic activity

Heterocyclic cores present in natural as well as synthetic world possess a diverse range of biological activities.[50-54] The current treatment regimen of amoebiasis is itself full of heterocyclic molecules, mainly azole based. An important review by Singh *et al*.[17] elaborated the synthesis and amoebicidal activities of a range of molecules from different sources. However, we will be restricting ourselves here mainly to molecules bearing azole moiety with excellent *in-vitro* activity profile. **Chart 2** shows some of the important recently reported synthetic azole based compounds possessing antiamoebic activity and their IC_{50} values are given in **Table ST1**

(*Supplementary file*). In the pursuit of novel molecule, recently three MNZ-thiosalicylate conjugates (**9a-c**, Chart 2) were reported.[26] These conjugates possessed IC_{50} value in the range of 0.015-0.028 μM, which was better than **1** (IC_{50} = 1.46 μM). The activity pattern of conjugates dictated that compound **9a** having no oxygen atom (linked to sulphur atom) showed least activity, while **9b** and **9c** having oxygen atom linked to sulphur atom demonstrated the best. This was attributed to the increased electron density on the molecule. The cytotoxic assay of compounds against MCF-7 cell line demonstrated their non-toxic nature in the concentration range of 2.5-250 μM.

The biological potential of hydrazones and chalcones are well established.[55-59] Drugs like dihydralazine, which is in use, is a well-known example of molecule having hydrazone moiety.[60] On the other hand, nature is a rich source of chalcone containing molecule.[61, 62] To explore the potential of these two moieties as antiamoebic agent, Azam and co-workers reported some hydrazone derivatives of **1** (**10a-b**, Chart 2) along with their *E. histolytica* inhibiton and cytotoxicity studies.[63] They found that the activating group like methoxy (**10b**, Chart 2) displayed better antiamoebic activity than compound having electron donating methyl group (**10a**, Chart 2). However, comparatively, both of them showed better efficiency than **1**. Chalcones (**11a-b**, Chart 2) were also found to be better inhibitors of *E. histolytica* than **1**.[64] The IC_{50} values for these two compounds were found to be 0.05 and 0.09 μM, which were much less than **1** (IC_{50} = 1.4 μM). Compound **11a** retaining two chlorine atoms displayed better antiamoebic activity than compound **11b** having a chlorine and a bromine atoms. Additionally, MTT assay against MCF-7 cell line depicts that these two compounds are non-toxic in concentration range 1.56-50 μM.

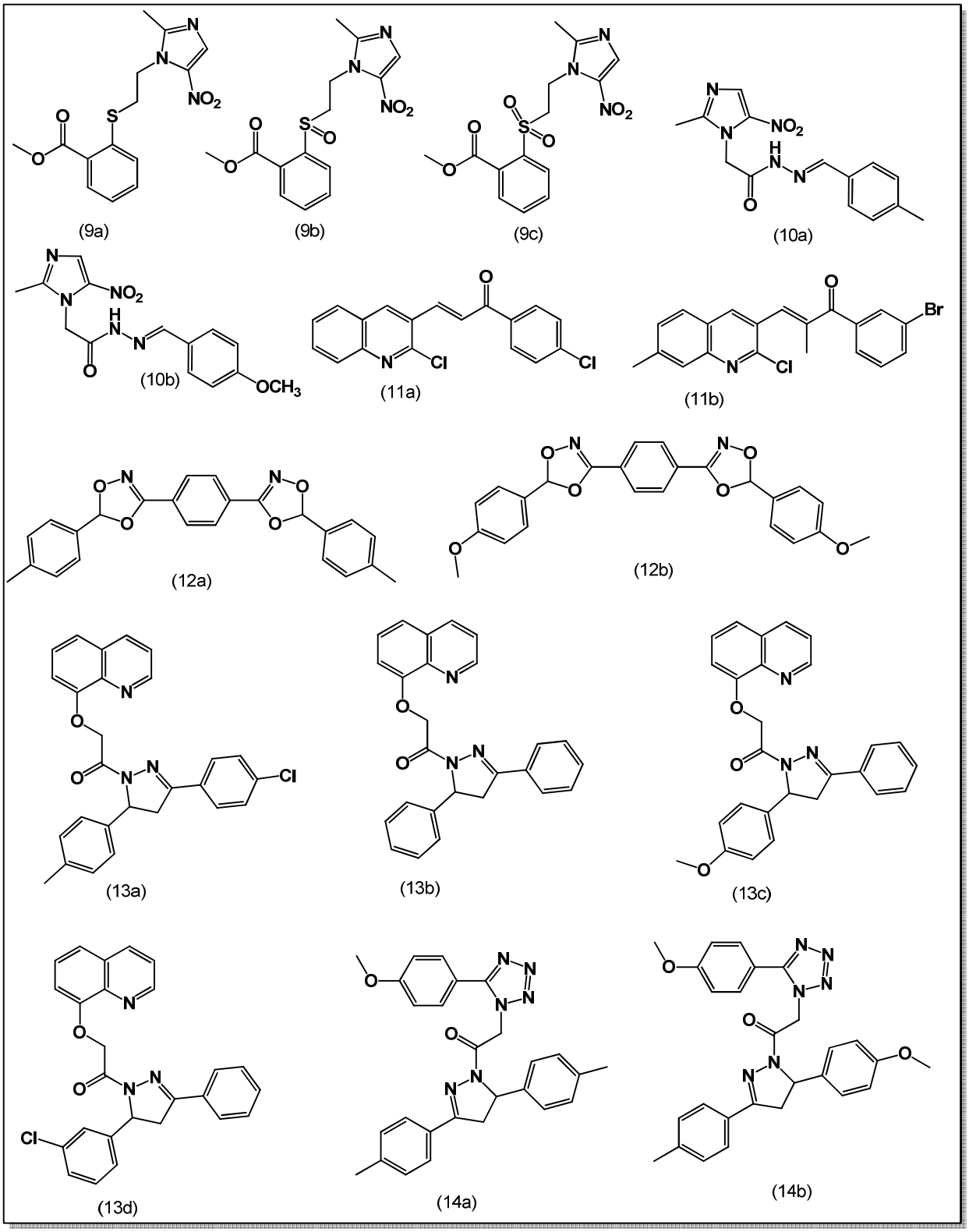
Dioxazole, bearing oxygen and nitrogen both, also displayed significant inhibitory activity against *E. histolytica*.[65] It has been reported that the inclusion of electron donating group like methyl and methoxy in a bisisoxazole backbone significantly improves the antiamoebic activity.[66] For example, compounds (**12a-b**, Chart 2) showed better activity than **1**. The IC_{50} values of these two compounds were 1.05 μM and 1.01 μM, respectively. Furthermore, the cytotoxicity assay on H9c2 cardiac myoblasts revealed non-cytotoxic nature of the compounds (viability 82% and 89%, respectively, at 12.5 μg/mL). Quinoline and pyrazoline both are important pharmacophores which exhibits a range of biological activities.[65, 67] In order to observe the synergistic effects of these two scaffolds, a series of quinoline based pyrazoline derivatives

(**13a-d**, Chart 2) were reported.[68] In the series of eleven compounds, four compounds showed potent antiamoebic activity. The IC_{50} values of the compounds **13a**, **13b**, **13c** and **13d** (Chart 2) were 0.05, 0.31, 0.06 and 0.29 μM, respectively which was much more pronounced than **1** (IC_{50} = 1.84 μM). Compound **13a** (Chart 2), having both electron donating and withdrawing groups showed best activity among the series. Compound **13b** (Chart 2), having no functional group showed slightly lower activity. However, compound **13c** (Chart 2) with electron donating methoxy group displayed second highest activity. Compound **13d** (Chart 2), having electron withdrawing group chlorine, showed moderate activity. All these compounds were non-toxic against MCF-7 cell line in the concentration range 1.56-50 μM.

In spite of popularity of tetrazole among medicinal chemist for various biological activities[69], no attempts have ever been made by any groups to assess its amoebicidal activity. Considering this, some new tetrazole-pyrazoline hybrids were reported by Azam and co-workers.[70] In the series of 15 compounds, four compounds (**14a-d**, Chart 2) showed good to moderate activity. IC_{50} values for compounds **14a-d** were found to be in the range of 0.86-1.20 μM; more potent than standard **1** (IC_{50} = 1.80 μM). In the previous examples, we saw that electronic factor is one of the governing factors for amoebicidal activity, same have been observed here. For instance, mild electron donating group (methyl) at *para* position of benzene rings attached to pyrazoline (**14a**, Chart 2) exhibited better activity than the rests. The cytotoxic assay against HepG2 cell line depicts non-toxic nature of the compounds in the concentration range of 3.13-25 μM. In another work, Rawat and co-workers[71] reported MNZ-triazole hybrids (**15a-d**, Chart 2) having IC_{50} values in the range of 0.008-0.08 μM. Especially the most active compound, 2-pyridyl-(1,2,3-triazolyl)metronidazole **(15d,** IC_{50} = 8.4 nm) seems to be very promising.

Thiazolidinone is one of the unique molecules, which contain all the three heteroatoms (N, O, S) in one ring.[72] It is known to display several pharmacological activities *viz*. antibacterial[73], fungicidal[74], antimicrobial[75, 76], antiproliferative[77], antiviral[78], anticonvulsant[79] and anticancer.[80-83] In the view of its intriguing importance as biologically active scaffold, a very first report on amoebicidal activities of a series of thiazolidinone **(16a-f)** was reported.[84] Screening of synthesized compounds against *E. histolytica* dictated that some of the molecules exhibited remarkable *in-vitro* activity and was better than **1**. The IC_{50} values of the compounds were found to be in the range of 0.11-0.64 μM. Among all the compounds in series, compound

16a showed lowest IC_{50} (0.11 μM). Cytotoxic assay on HepG2 cell line showed non-toxic nature of the compounds in the concentration range of 3.13-25 μM.



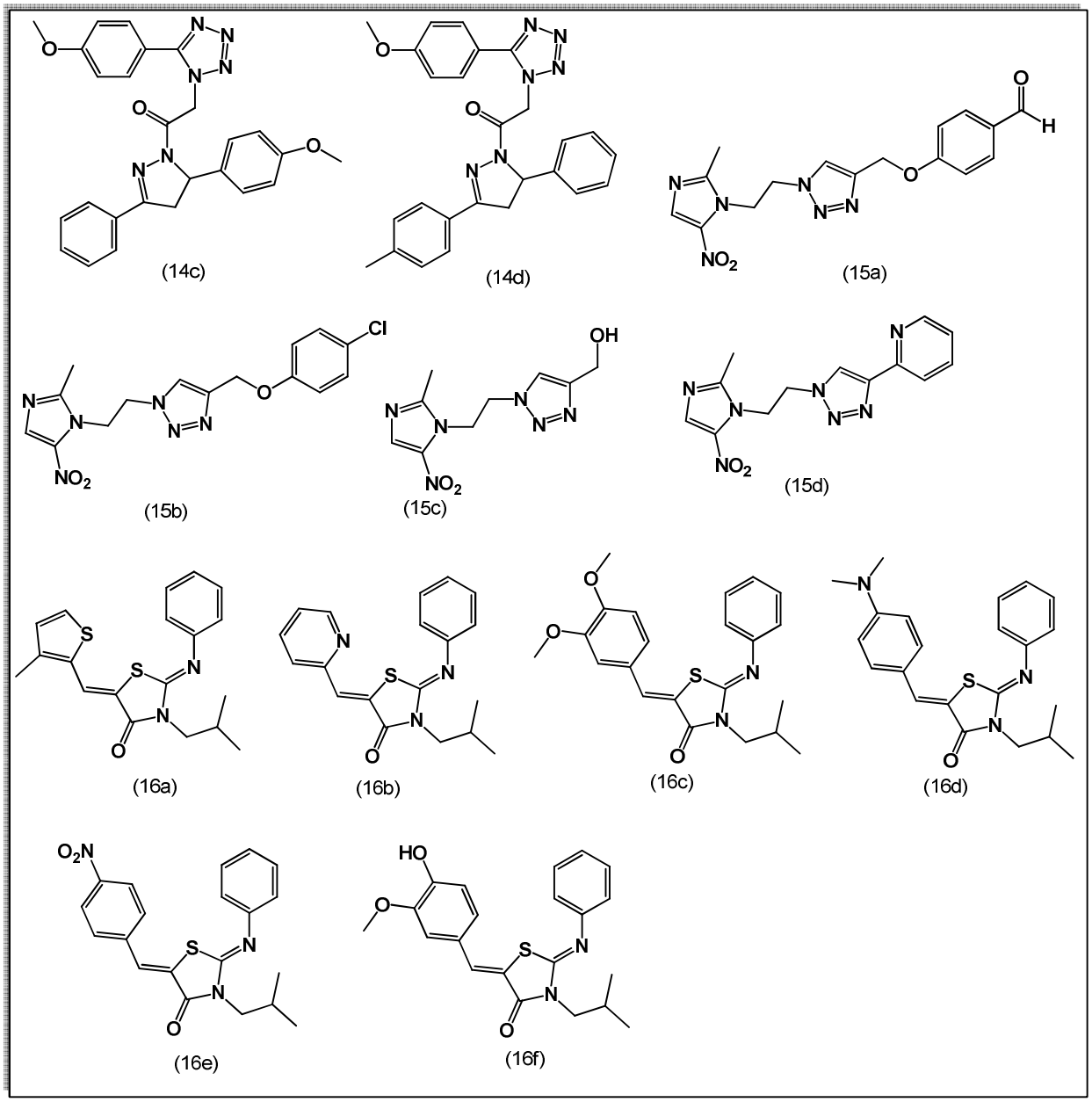


Chart 2: Structure of recently reported antiamoebic agents of azole family.

5. Correlation between drug-likeness-rule of five (Ro5) and *in-vitro* activity

The study of drug-likeness gives an idea about the possibility of whether a molecule could act as drug or not and is a very useful tool for the drug development.[85-87] It has been reported, at least in many cases, molecules obeying the "Lipinski's rules of Five (Ro5)" is likely to behave as drug. According to the first rule, an ideal drug candidate should have logP ≤ 5, where logP is octanol-water partition co-efficient and describes the ability of a compound to dissolve into hydrophobic

(non-aqueous) medium. Hydrobhobicity is compulsory for the drug permeation through various biological membranes and affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism and toxicity of the molecules. The second rule states that the molecule should have molecular weight less than 500. Third and fourth rule deals with number of H-bonding. Accordingly, a molecule should possess less than ten hydrogen bond acceptor (HBA) units and less than five hydrogen bond donor (HBD) units.[87, 88] In addition to these, some extensions like polar surface area (PSA), numbers of rotatable bonds (RB) etc. were also added to maximize the accuracy of prediction of drug bioavailability.[89] Poor absorption or permeation is likely to happen if a molecule violates two or more of these rules.[90]

To check the possibility of discussed molecules as antiamoebic *drug/s*, we carried out an extensive study based on Ro5. The outcomes of the study for reported molecules as well as standards are summarized in **Table ST1** (*Supplementary file*). The data given in table suggests that, except few, all other compounds follow Lipinski's parameters and are potential candidates for further clinical studies. Among the reported synthetic molecules, **9a** showed logP, HBA, HBD & MW of 2.4, 4, 0 and 321.3, respectively, while for compounds **9b**, these values were logP = 0.92, HBA = 5; HBD = 0 & MW = 337.3 and for **9c** it was logP = 1.03, HBA = 6, HBD = 0 and MW = 353.3. Upon inclusion of one oxygen to the S-atom in **9a**, a significantly decrease in the partition co-efficient and increase in MW and HBA was observed in **9b**. The pattern of activity (**9b** > **9c** > **9a**) among the compounds is strongly supporting the applicability of Ro5. Similar results were observed for compounds **15c** and **15d**, which showed activity in nanomolar concentration range. All these values along with their biological results dictate the possibility of these molecules as future antiamoebic drug and, thus, *in-vivo* studies should be carried out.

Since it is easy to visualize and analyze data than other methods[91], we herein, also including a table (**Table 1**) showing drug property data in the form of simple color and shape. From the Table, it is clear that all new compounds (**9-16**) including standard drugs (except **8**, which is a natural product and falls under exception for Ro5) reasonably follow the criteria needed for a drug candidate. The number of RBs, which is also as a detrimental factor in the oral bioavailability, was under the upper limit. It has been reported that for a molecule to possess ≥ 20% oral bioavailability, it should not have more than 13 RBs.[92] Similarly PSA, of which cut off value was ≤ 140 $Å^2$ (when RB ≤ 10) for ≥ 20% oral bio-availability[93] was within the limit.

Table 1. Representative drug property data with examples of simple colour and shape highlighting: polar surface area (PSA) values as horizontal bars; molecular weight (Mol. Wt.) with graphical pies and grey-scale shading; log P with green-yellow-red coloring; and rotatable bond count with colouring if value is > 8.[91] Small horizontal bars and light color of the properties indicates more drug-likeness.

Drug	PSA	Mol Wt	logP	Rotatable bonds
1	83.9	171.2	-0.46	3
2	97.8	247.3	-0.58	5
3	83.9	219.6	0.26	4
4	83.9	185.2	-0.04	3
5	52.2	480.6	4.49	7
6	33.1	397.0	3.69	0
7	59.8	328.2	3.08	5
8	347.3	615.6	-8.31	9
9a	89.9	321.3	2.40	7
9b	107.0	337.3	0.92	7
9c	124.1	353.3	1.03	7
10a	105.1	301.3	1.55	5
10b	114.3	317.3	0.88	6
11a	30.0	328.2	5.48	3
11b	30.0	400.7	6.55	3
12a	61.6	400.4	7.07	4
12b	80.1	432.4	5.73	6
13a	54.8	455.9	5.69	5
13b	54.8	407.5	4.57	5
13c	64.0	437.5	4.42	6
13d	54.8	441.9	5.18	5
14a	85.5	466.5	4.85	6
14b	94.7	482.5	4.18	7
14c	94.7	468.5	3.66	7
14d	85.5	452.5	4.33	6
15a	120.7	356.3	1.44	8
15b	103.6	362.8	2.33	7
15c	114.6	252.2	-0.61	5
15d	107.2	299.3	1.28	5
16a	32.7	356.5	5.81	4
16b	45.6	337.4	4.40	4
16c	51.1	396.5	5.06	6
16d	35.9	379.5	5.49	5
16e	78.5	381.5	5.32	5
16f	62.1	382.5	4.92	5

6. Relationship between frontier molecular orbitals (FMO) and IC_{50} value of the compounds

We already discussed the Ro5 to predict the future of recently reported azole based molecules as drug candidates. To understand these properties further at supra-molecular level, we carried out frontier molecular orbitals (FMO) studies to ensure the effect of electronic distribution within molecule on biological activities (in this case, antiamoebic). It is important to note that molecules having smaller interfrontier orbitals are chemically more active and have lower kinetic stability.[94-96] The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of a molecule give significant information about the potency of biological activity. The ionization enthalpy is related to HOMO while the LUMO corresponds to electron affinity (electron gain enthalpy) of a molecule.[97] The HOMO and LUMO energy values along with chemical potential and hardness of the standards (**1-8**, Chart 1) and recently reported azole-based molecules (**9-16**, Chart 2) are given in **Table ST2** (*Supplementary file*). **Figure SF1** (*Supplementary file*) depicts orbital diagrams of **1-16** (Chart 1 & 2). The chemical potential of standard drugs (**1-8,** Chart 1) ranged from -5.69 to -4.91 eV. Interestingly, for recently reported azole-based molecules (**9-16**, Chart 2) this range was -5.63 to -4.47 eV, which is much similar to the standard drugs. Adding to this, when we compared hardness of the standard drugs (**1-8,** Chart 1) with reported azole-based molecules (**9-16**, Chart 2), they were also very much similar (-4.48 to -3.11 eV for **1-8** *vs* -4.38 to -2.96 eV for **9-16**). When we tried to correlate activity of the compounds with the negative highest electron affinity,[98] we found that compounds **9c**, **15b**, **15c**, and **15d** have highest affinity (-1.26, -1.32, -1.27 and -1.22 eV, respectively) and hence they should possess potent amoebicidal activity. In fact, we observe the same. Compounds **15a-d** reported by Rawat and co-workers[71] and compounds **9a-c** reported by Azam and co-workers[26] possessed excellent in-vitro antiamoebic activity. **Fig. 2** depicts HOMO and LUMO orbitals of a standard drug **1** (MNZ) and active synthetic compounds **9b** and **15d**. Figure 2 and **Figure SF1** (*supplementary file*) indicates that the HOMO-LUMO undergoes a shift from imidazole ring to the other rings upon derivatization, resulting in a dramatic change in biological activity of the molecules. In most of the active compounds (*viz*. **1**, **9b**, and **15d**), the LUMO level was mainly distributed over 2-methyl 5-nitro imidazole ring, which is the backbone to express antiamoebic activity. This implies that the new MNZ based derivatives shows variation in IC_{50} value due to shifting of LUMO levels between

2-methyl 5-nitro imidazole ring and other aromatic rings carrying different functionality. Furthermore, due to this, a variation in nitro group reduction affinity cannot be ruled out.

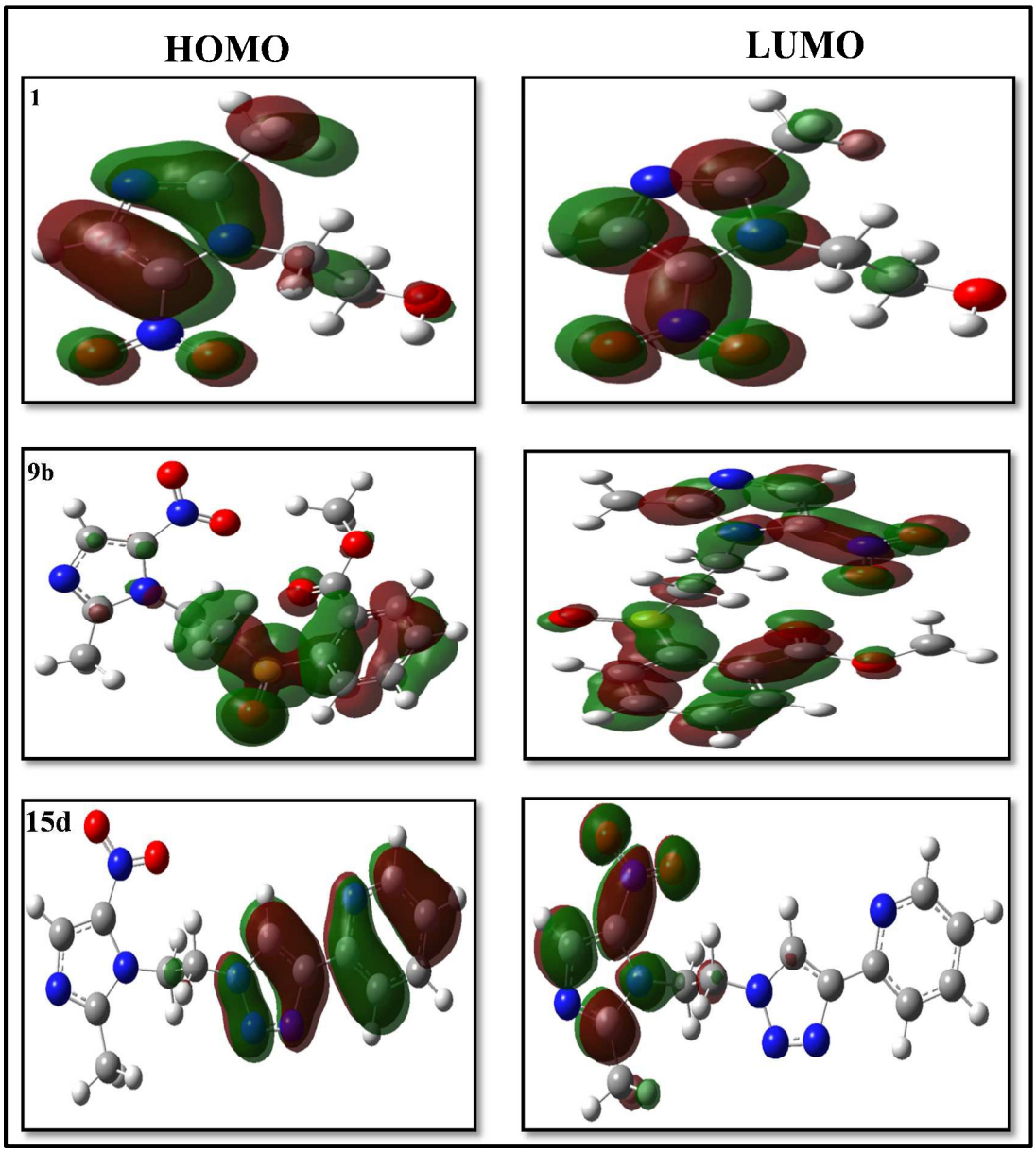


Fig. 2 HOMO-LUMO molecular orbitals of MNZ (**1**) and most active compounds (**9b**) and (**15d)**

Furthermore, we draw a plot between IC_{50} and LUMO energies (**Fig. 3**), to predict and set a limit for LUMO energy level, which a molecule should possess in order to show better activity. From figure, it is interesting to note that majority of the molecules, which we discussed in this paper (**1-16**), displayed LUMO energy between -1.34 to -0.54 eV. Thus, this value could be treated as limit while designing new antiamoebic agent.

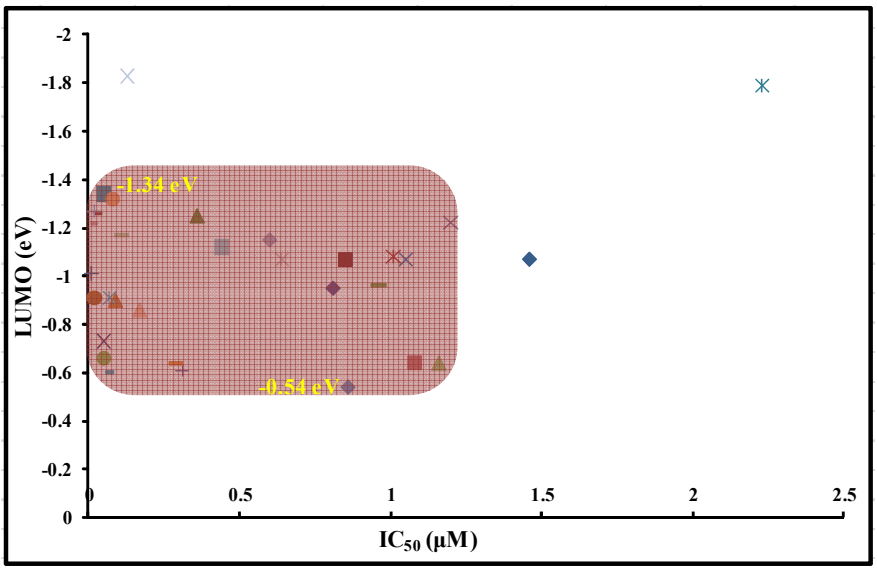


Fig. 3. Relation between LUMO energy and IC_{50} values of the hit compounds.

Conclusion

Amoebiasis is one of the silent killers in developing and under-developed countries. However, the number of deaths caused by this disease is under the control, but it causing a considerable loss of health and economy. In addition to the current therapeutic regime, several azole-based compounds have been reported with activity better than the standards. We have reviewed the potential of some new synthetic azole-based molecules as antiamoebic agents. Through theoretical studies, we observed that some of the newly reported azole based compounds deserve further clinical studies as their physico-chemical properties are similar or comparable with the currently employed drugs. DFT studies indicated that LUMO energy level should lie between -1.34 to -0.54 eV to show excellent activity. The HOMO-LUMO undergoes a shift from imidazole ring to the other rings upon derivatization, resulting in a dramatic change in biological activity of the molecules. In most of the active compounds, the LUMO level was mainly distributed over 2-methyl 5-nitro imidazole ring. Among the discussed, compounds **9a-c** and **15b-d** need special attention from the people working in this area. They obeyed the rules of drug likeliness to all extents. Hence, based on experimental *in-vitro* results and theoretical calculations, we strongly support further *in-vivo* studies of the above-said compounds to introduce new antiamoebic agents in the market.

Acknowledgement

The author (MM) is highly thankful to Mr. Jawad Ahmad Siddiqui (Chairman), Al-Falah University, for his co-operation and motivation during the preparation of this article.

References

1. N. Nagata, T. Shimbo, J. Akiyama, R. Nakashima, S. Nishimura, T. Yada, K. Watanabe, S. Oka and N. Uemura, *Emerging infectious diseases*, 2012, **18**, 717.
2. W. Stauffer and J. I. Ravdin, *Current opinion in infectious diseases*, 2003, **16**, 479-485.
3. J. I. Ravdin, *Clinical Infectious Diseases*, 1995, 1453-1464.
4. T. E. Bercu, W. A. Petri and B. W. Behm, *Current gastroenterology reports*, 2007, **9**, 429-433.
5. M. Mushtaque, *European journal of medicinal chemistry*, 2015, **90**, 280-295.
6. L. Mortimer and K. Chadee, *Experimental parasitology*, 2010, **126**, 366-380.
7. O. Addib, H. Ziglam and P. J. Conlong, *The Libyan Journal of Medicine*, 2007, **2**, 214-215.
8. K. Ohnishi and M. Murata, *Epidemiology and infection*, 1997, **119**, 363-367.
9. M. A. Alam, A. Maqbool, M. M. Nazir, M. Lateef, M. S. Khan, A. N. Ahmed, M. Ziaullah and D. S. Lindsay, *Journal of Parasitology*, 2015, **101**, 236-239.
10. S. Ibrahim, O. El-Matarawy, M. Ghieth, E. Abu Sarea and A. El-Badry, *World J Microbiol Biotechnol*, 2015, **31**, 385-390.
11. J. Nath, N. Banyal, D. S. Gautam, S. K. Ghosh, B. Singha and J. Paul, *Epidemiology & Infection*, 2015, **143**, 108-119.
12. S. Shanan, H. Abd, M. Bayoumi, A. Saeed and G. Sandström, *BioMed research international*, 2015, **2015**.
13. S. J. van Hal, D. J. Stark, R. Fotedar, D. Marriott, J. T. Ellis and J. L. Harkness, 2007.
14. S. L. Stanley, *The Lancet*, 2003, **361**, 1025-1034.
15. D. Moncada, K. Keller and K. Chadee, *Infection and immunity*, 2003, **71**, 838-844.
16. C. Sundaram, B. Prasad, G. Bhaskar, V. Lakshmi and J. Murthy, *JOURNAL-ASSOCIATION OF PHYSICIANS OF INDIA.*, 2004, **52**, 251-252.
17. S. Singh, N. Bharti and P. P. Mohapatra, *Chemical reviews*, 2009, **109**, 1900-1947.
18. J. Kirchmair, A. H. Goller, D. Lang, J. Kunze, B. Testa, I. D. Wilson, R. C. Glen and G. Schneider, *Nat Rev Drug Discov*, 2015, **14**, 387-404.
19. J. A. Upcroft, R. W. Campbell, K. Benakli, P. Upcroft and P. Vanelle, *Antimicrobial Agents and Chemotherapy*, 1999, **43**, 73-76.
20. T. Mukherjee and H. Boshoff, *Future medicinal chemistry*, 2011, **3**, 1427-1454.
21. A. Azam and S. Agarwal, *Current Bioactive Compounds*, 2007, **3**, 121-133.
22. D. Leitsch, A. G. Burgess, L. A. Dunn, K. G. Krauer, K. Tan, M. Duchêne, P. Upcroft, L. Eckmann and J. A. Upcroft, *Journal of antimicrobial chemotherapy*, 2011, **66**, 1756-1765.
23. D. Leitsch, D. Kolarich, I. Wilson, F. Altmann and M. Duchêne, *PLoS Biol*, 2007, **5**, e211.
24. M. Müller, *Biochemical pharmacology*, 1986, **35**, 37-41.
25. S. N. Moreno and R. Docampo, *Environmental Health Perspectives*, 1985, **64**, 199-208.
26. A. Salahuddin, S. M. Agarwal, F. Avecilla and A. Azam, *Bioorganic & medicinal chemistry letters*, 2012, **22**, 5694-5699.
27. H. B. Fung and T.-L. Doan, *Clinical therapeutics*, 2005, **27**, 1859-1884.

28. D. Bansal, N. Malla and R. Mahajan, *Indian Journal of Medical Research*, 2006, **123**, 115.
29. R. S. Gupta and L. Siminovitch, *Cell*, 1977, **10**, 61-66.
30. E. S. Akinboye and O. Bakare, *Open Natural Products Journal*, 2011, **4**, 8-15.
31. R. Haque, C. D. Huston, M. Hughes, E. Houpt and W. A. Petri, *New England Journal of Medicine*, 2003, **348**, 1565-1573.
32. R. Knight, *Journal of antimicrobial chemotherapy*, 1980, **6**, 577-593.
33. S. J. Powell, *Bulletin of the World Health Organization*, 1969, **40**, 953-956.
34. J. Samuelson, A. Burke and J. Courval, *Antimicrobial Agents and Chemotherapy*, 1992, **36**, 2392-2397.
35. K. Kapoor, M. Chandra, D. Nag, J. Paliwal, R. Gupta and R. Saxena, *International journal of clinical pharmacology research*, 1998, **19**, 83-88.
36. M. Khaw and C. B. Panosian, *Clinical microbiology reviews*, 1995, **8**, 427-439.
37. F. Calzada, J. A. Cervantes-Martínez and L. Yépez-Mulia, *Journal of ethnopharmacology*, 2005, **98**, 191-193.
38. *http://www.drugs.com/sfx/metronidazole-side-effects.html last accessed 26/09/2015*.
39. D. W. Kim, J.-M. Park, B.-W. Yoon, M. J. Baek, J. E. Kim and S. Kim, *Journal of the neurological sciences*, 2004, **224**, 107-111.
40. S. Toumi, M. Hammouda, A. Essid, L. Medimagh, L. B. Slamia and C. Laouani-Kechrid, *Medecine et maladies infectieuses*, 2009, **39**, 906-908.
41. V. Purohit and A. K. Basu, *Chemical research in toxicology*, 2000, **13**, 673-692.
42. G. Elizondo, M. E. Gonsebatt, A. M. Salazar, I. Lares, P. Santiago, J. Herrera, E. Hong and P. Ostrosky-Wegman, *Mutation Research/Genetic Toxicology*, 1996, **370**, 75-80.
43. N. Samarawickrema, D. Brown, J. Upcroft, N. Thammapalerd and P. Upcroft, *Journal of antimicrobial chemotherapy*, 1997, **40**, 833-840.
44. *http://www.drugsupdate.com/generic/view/ 464/Secnidazole, last accessed 26/09/2015*.
45. *http://www.medindia.net/doctors/drug_information/secnidazole.htm, last accessed 26/09/2015*.
46. R. Lemmens-Gruber, A. Karkhaneh, C. Studenik and P. Heistracher, *British journal of pharmacology*, 1996, **117**, 377-383.
47. D. I. Edwards, *Biochemical pharmacology*, 1986, **35**, 53-58.
48. P. Lanzky and B. Halting-Sørensen, *Chemosphere*, 1997, **35**, 2553-2561.
49. N. Hammami, C. Drissi, R. Sebai, M. Araar, Y. Maatallah, L. Belghith, S. Nagi, F. Hentati and M. B. Hamouda, *Journal of neuroradiology*, 2007, **34**, 133-136.
50. R. Dua, S. Shrivastava, S. Sonwane and S. Srivastava, *Advances in Biological Research*, 2011, **5**, 120-144.
51. I. Ali, A. Haque, K. Saleem and M. F. Hsieh, *Bioorganic & medicinal chemistry*, 2013, **21**, 3808-3820.
52. K. Saleem, W. A. Wani, A. Haque, M. N. Lone, M.-F. Hsieh, M. A. Jairajpuri and I. Ali, *Future medicinal chemistry*, 2013, **5**, 135-146.
53. I. Ali, S. K. Rahisuddin, A. Haque and A. El-Azzouny, *Egypt Pharma J*, 2010, **9**, 133-179.
54. I. Ali, W. A. Wani, A. Khan, A. Haque, A. Ahmad, K. Saleem and N. Manzoor, *Microbial pathogenesis*, 2012, **53**, 66-73.
55. S. Bala, G. Uppal, A. Kajal, S. Kamboj and V. Sharma, *International Journal of Pharmaceutical Sciences Review and Research*, 2013, **18**, 65-74.

56. G. Verma, A. Marella, M. Shaquiquzzaman, M. Akhtar, M. R. Ali and M. M. Alam, *Journal of pharmacy & bioallied sciences*, 2014, **6**, 69.
57. Z. Nowakowska, *European journal of medicinal chemistry*, 2007, **42**, 125-137.
58. S. Rollas and S. G. Küçükgüzel, *Molecules*, 2007, **12**, 1910-1939.
59. M. J. Matos, S. Vazquez-Rodriguez, E. Uriarte and L. Santana, *Expert opinion on therapeutic patents*, 2015, **25**, 351-366.
60. , 2015.
61. Z. Rozmer and P. Perjési, *Phytochemistry Reviews*, 2014, 1-34.
62. A. Leon-Gonzalez, N. Acero, D. Munoz-Mingarro, I. Navarro and C. Martin-Cordero, *Current medicinal chemistry*, 2015.
63. M. Y. Wani, A. R. Bhat, A. Azam and F. Athar, *European journal of medicinal chemistry*, 2013, **64**, 190-199.
64. F. Hayat, E. Moseley, A. Salahuddin, R. L. Van Zyl and A. Azam, *European journal of medicinal chemistry*, 2011, **46**, 1897-1905.
65. A. R. Bhat, F. Athar and A. Azam, *European journal of medicinal chemistry*, 2009, **44**, 926-936.
66. P. F. Iqbal, H. Parveen, A. R. Bhat, F. Hayat and A. Azam, *European journal of medicinal chemistry*, 2009, **44**, 4747-4751.
67. M. Abid, A. R. Bhat, F. Athar and A. Azam, *European journal of medicinal chemistry*, 2009, **44**, 417-425.
68. F. Hayat, A. Salahuddin, S. Umar and A. Azam, *European journal of medicinal chemistry*, 2010, **45**, 4669-4675.
69. P. Mohite and V. Bhaskar, *Int J Pharm Tech Res*, 2011, **3**, 1557-1566.
70. M. Y. Wani, A. R. Bhat, A. Azam, D. H. Lee, I. Choi and F. Athar, *European journal of medicinal chemistry*, 2012, **54**, 845-854.
71. B. Negi, K. K. Raj, S. M. Siddiqui, D. Ramachandran, A. Azam and D. S. Rawat, *ChemMedChem*, 2014, **9**, 2439-2444.
72. A. Verma and S. K. Saraf, *European journal of medicinal chemistry*, 2008, **43**, 897-905.
73. P. Kenderekar, R. Siddiqui, P. Patil, S. Bhusare and R. Pawar, *Indian journal of pharmaceutical sciences*, 2003, **65**, 313-315.
74. H.-L. Liu, Z. Lieberzeit and T. Anthonsen, *Molecules*, 2000, **5**, 1055-1061.
75. P. Vicini, A. Geronikaki, K. Anastasia, M. Incerti and F. Zani, *Bioorganic & medicinal chemistry*, 2006, **14**, 3859-3864.
76. S. Wang, Y. Zhao, G. Zhang, Y. Lv, N. Zhang and P. Gong, *European journal of medicinal chemistry*, 2011, **46**, 3509-3518.
77. V. Gududuru, E. Hurh, J. T. Dalton and D. D. Miller, *Bioorganic & medicinal chemistry letters*, 2004, **14**, 5289-5293.
78. J. Balzarini, B. Orzeszko-Krzesińska, J. K. Maurin and A. Orzeszko, *European journal of medicinal chemistry*, 2009, **44**, 303-311.
79. A. Agarwal, S. Lata, K. Saxena, V. Srivastava and A. Kumar, *European journal of medicinal chemistry*, 2006, **41**, 1223-1229.
80. D. Havrylyuk, L. Mosula, B. Zimenkovsky, O. Vasylenko, A. Gzella and R. Lesyk, *European journal of medicinal chemistry*, 2010, **45**, 5012-5021.
81. D. Havrylyuk, B. Zimenkovsky, O. Vasylenko, L. Zaprutko, A. Gzella and R. Lesyk, *European journal of medicinal chemistry*, 2009, **44**, 1396-1404.

82. D. Kaminskyy, B. Zimenkovsky and R. Lesyk, *European journal of medicinal chemistry*, 2009, **44**, 3627-3636.
83. I. Subtel'na, D. Atamanyuk, E. Szymańska, K. Kieć-Kononowicz, B. Zimenkovsky, O. Vasylenko, A. Gzella and R. Lesyk, *Bioorganic & medicinal chemistry*, 2010, **18**, 5090-5102.
84. M. Mushtaque, F. Avecilla and A. Azam, *European journal of medicinal chemistry*, 2012, **55**, 439-448.
85. C. A. Lipinski, *Drug Discovery Today: Technologies*, 2004, **1**, 337-341.
86. C. A. Lipinski, *Journal of pharmacological and toxicological methods*, 2000, **44**, 235-249.
87. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Advanced drug delivery reviews*, 2012, **64**, 4-17.
88. S. K. Mondal, N. B. Mondal, S. Banerjee and U. K. Mazumder, *Indian Journal of Pharmacology*, 2009, **41**, 176-181.
89. T. H. Keller, A. Pichota and Z. Yin, *Current opinion in chemical biology*, 2006, **10**, 357-361.
90. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Advanced drug delivery reviews*, 2001, **46**, 3-26.
91. T. J. Ritchie, P. Ertl and R. Lewis, *Drug discovery today*, 2011, **16**, 65-72.
92. S. Tian, Y. Li, J. Wang, J. Zhang and T. Hou, *Molecular pharmaceutics*, 2011, **8**, 841-851.
93. D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward and K. D. Kopple, *Journal of medicinal chemistry*, 2002, **45**, 2615-2623.
94. D. Lewis, C. Ioannides and D. Parke, *Xenobiotica*, 1994, **24**, 401-408.
95. B. Kosar and C. Albayrak, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2011, **78**, 160-167.
96. M. J. Alam and S. Ahmad, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2015, **136**, 961-978.
97. T. Koopmans, *Physica*, 1934, **1**, 104-113.
98. A. K. Bhattacharjee, D. J. Skanchy, B. Jennings, T. H. Hudson, J. J. Brendle and K. A. Werbovetz, *Bioorganic & medicinal chemistry*, 2002, **10**, 1979-1989.