Send Orders for Reprints to reprints@benthamscience.ae

Letters in Drug Design & Discovery, 2018, *15*, 887-894

RESEARCH ARTICLE

Antihelminthic Activity of Some 2-substituted Thieno[2,3-*d*]pyrimidin-4-ones

Anelia Mavrova[a], Stefan Dimov[a], Dimitar Vuchev[b], Kameliya Anichina[a] and Denitsa Yancheva[c,*]

[a]*Department of Organic Synthesis, University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria;* [b]*Section of Parasitology and Tropical Deseases, Medicinal University, Plovdiv, Bulgaria;* [c]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Build. 9, 1113, Sofia, Bulgaria*

Abstract: ***Background*:** One of the successful approaches for rational design of bioactive compounds is the bioisosterism strategy. Thus, the bioisosteric relation of thieno[2,3-*d*]pyrimidin-4-ones with quinazolines, cytosine and uracil resulted in the generation of compounds with variety of biological properties including antiparasitic activity. In contrast to mammals, all parasites are unable to synthesize purines de novo and rely instead on the Purine Salvage Pathway (PSP) to obtain purines, which are essential for their survival. Having in view the above mentioned facts we designed and synthesized some thieno[2,3-*d*]pyrimidines as bioisosters in order to evaluate their antitrichinellosis efficacy.

***Methods*:** The target compounds were synthesized by one-pot cyclocondensation reaction passing dry hydrogen chloride gas through a solution of 2-aminothiophene-carboxylate and a series of alkyl respectively aryl nitriles as precursors.

The parasitological screening *in vitro* was carried out by using encapsulated infective larvae of *Trichinella spiralis*, 100 speciments per 1 mL physiological solution, released in advance from the muscle capsules by digestion with acid pepsin solution.

***Results*:** Fourteen 2-substituted-thieno[2,3-*d*]pyrimidin-4-ones were synthesized and their structure was identified. The data obtained by the antitrichinellosis screening showed that compounds **2**, **8** and **15** exhibit higher activity than the reference drugs *albendazole* and *ivermectin* at concentrations of 0.37, 0.35 and 0.48 μM resp. 0.92, 0.88 and 1.2 μM after 24-hour incubation of the samples. The highest efficacy at both incubations was revealed by 2-benzyl-5,6,7,8-tetrahydrothieno[2,3-*d*]pyrimidin-4(3H)-one **2** - 97.94% and 100%, respectively. Compound **15** demonstrated 97.5% antiparasitic activity after 48h at concentration of 250 μg/ml. The theoretically calculated intestinal absorption (%ABS) of the tested thieno[2,3-*d*]pyrimidines displayed higher values than that of *albendazole.* The structure-activity relationship (SAR) data of the tested compounds **2**, **4**-**8** and **15** complies with the Lipinski's rule. It was assumed that the low molecular volume (Vol.) and logP of compound **15** led to enhancement of the compound activity by increasing the penetration through the ion channels of the parasitic cells as it was observed in some *albendazole* derivatives**.**

Conclusion: The tested thienopyrimidin-4-ones possess higher anthelminthic effect than *albendazole* against *Trichinella spiralis*. Compounds **2**, **4**-**8** and **15** demonstrated higher absorption %ABS than *albendazole*. According to the SAR analysis, the partition coefficient (logP) is essential for parasite drug penetration due to diffusion through the cell membrane.

ARTICLE HISTORY

Received: June 22, 2017
Revised: October 13, 2017
Accepted: October 17, 2017

DOI:
10.2174/1570180814666171027161555

Keywords: Thieno[2,3-d]pyrimidines, bioisosterism, SAR, antiparasitic activity, *Trichinella spiralis*, Lipinski's rule.

1. INTRODUCTION

The bioisosterism strategy is one of the successful approaches for rational design of bioactive compounds. The process of molecular modification using a lead compound as starting point is mostly focused on the synthesis of novel derivatives, based on the knowledge for the structural factors and the physico-chemical properties of all its pharmacophoric groups. The term bioisosteretism is introduced in order to explain the revealed similar or antagonistic biological effects of structurally related compounds. Later, the bioisosteres were defined as a subgroup or molecules with similar

*Address correspondence to this author at the Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Build. 9, 1113, Sofia, Bulgaria; Tel: 0035929606106; Fax: 0035928700225; E-mail deni@orgchm.bas.bg

1570-1808/18 $58.00+.00

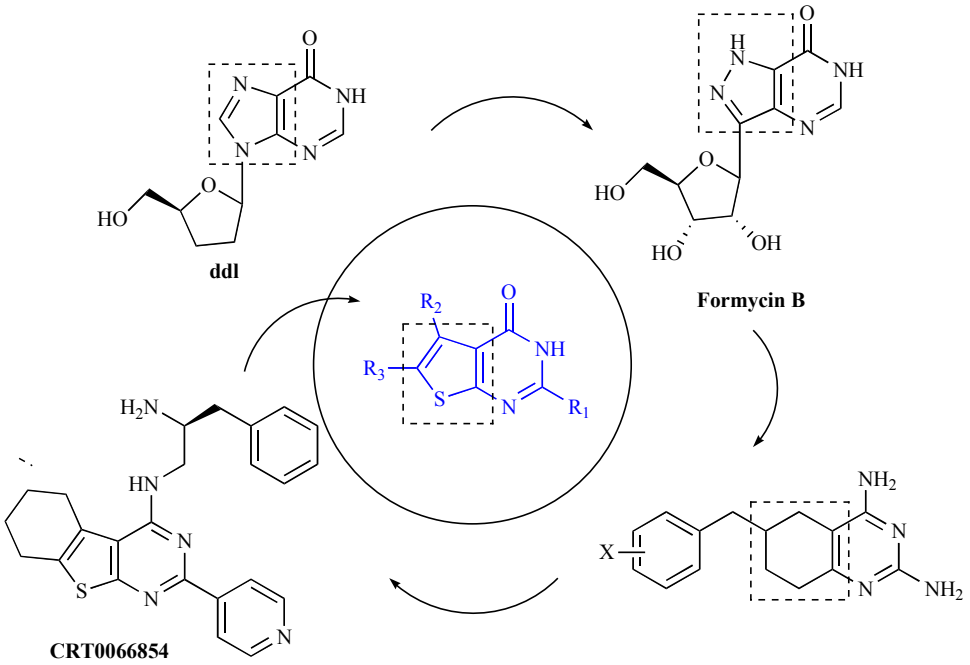


Fig. (1). Bioisosterism between pyrimidine derivatives.

physico-chemical properties exhibiting similar or antagonistic biological activities [1-2]. Thus bioisosteric relation of thieno[2,3-*d*]pyrimidin-4-ones with quinolones, cytosine and uracil led to the generation of variety of biological properties as antibacterial [3], antiparasitic [4], analgesic [5], anti-inflammatory [5], anticancer [6], antioxidant [7] and antiemetic [8].

Parasites are the cause of a number of major human and animal diseases. Parasites release a variety of molecules that help them to penetrate the defensive barriers and avoid the immune attack of the host. Fortunately, both protozoan and metazoan, parasites seem to share specificity to the purine metabolism. In contrast to mammals, all parasites are unable to synthesize purines de novo and rely instead on the Purine Salvage Pathway (PSP) to obtain purines, which are essential for their survival. Furthermore, the resistance against chemotherapy has been spreading for many years and for that reason chemotherapy must predicate on specific targets [9, 10].

Some purine derivatives (Fig. **1**) such as 2′,3′-dideoxyinosine (ddI) [11] were shown to be active against *T. gondii in vitro* and in a toxoplasmic encephalitis model in mice as well as formycin B [12], active against *C. parvum in vitro*. A number of quinazolines such as lapatinib, a tyrosine kinase and DHFR inhibitor, exhibit excellent parasitic efficacy. These results provide a guideline for the study of other derivatives. The bioisosterism studies between the structure of quinazoline and thienopyrimidine derivatives have shown that replacement of the aromatic ring with thiophene heterocycle leads to an increase in antiparasitic activity. A number of thieno[2,3-*d*]pyrimidines having TK, DHFR and PKC inhibitory activity have been reported in the literature [13].

Trichinella spiralis secretes, among others, protein kinases and phosphatases, endonucleases, and DNA-binding proteins, which are all thought to interfere with the host cellular signals for muscle cell differentiation [14-17]. PKC (protein kinase C) is an important family of serine/threonine protein kinase isoenzymes that contribute to many different cellular and tissue functions such as transmission of cell proliferation signals, apoptosis and angiogenesis. PKC is activated in response to multiple signals, such as Vascular Endothelial Growth Factor (VEGF) [18]. Thieno[2,3-d]pyrimidine derivatives having protein kinase inhibitory activity as the selective inhibitor of the aPKC isoenzymes CRT0066854 have been reported in the literature [19]. That suggests the manifestation of antiparasitic activity by inhibiting the activation of VEGF, thus preventing the induction of angiogenesis and feeding the parasitic capsule.

Considering this structural bioisosterism to purine and quinazoline derivatives it can be assumed that thieno[2,3-*d*]pyrimidin-4-ones also could possess antiparasitic or anti-helminthic activity. On the basis of the above mentioned facts, we were motivated to design and synthesize some thieno[2,3-*d*]pyrimidin-4-ones in order to study their anti-helminthic activity against nematode parasite *Trichinella spiralis*.

2. EXPERIMENTAL

2.1. Materials and Methods

Melting points (mp) were determined on an Electrothermal AZ 9000 3MK4 apparatus and are uncorrected. IR spectra were recorded on a Bruker spectrometer as potassium bromide disks. ^{1}H-NMR spectra were recorded on a Bruker Avance II+ 250 MHz and a Bruker Avance II+ 600 MHz NMR instrument. Chemical shifts were expressed relative to Tetramethylsilane (TMS) and were reported as δ (ppm). The coupling constants are given in Hz. The measurements were carried out at ambient temperature (300 K). Thin Layer Chromatography (TLC, R_f

values) was performed on silica gel plates (Merck, 0.2 mm thick).

2.2. Chemistry

The ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate **1** was synthesized according to the earlier published methods by Gewald [20].

2.2.1. General Procedure for the Synthesis of thieno[2,3-d]pyrimidin-4-ones 2-14

Compound **1** (0.0094 mol) and the appropriate cyano derivative R-CN (0.018 mol) were dissolved in 10 ml dioxane in a round-bottom flask. Dry hydrogen chloride gas was passed through the solution for 6 h. This reaction mixture was allowed to stay 12 h at room temperature. It was then poured into a beaker containing crushed ice and 10% NH_4OH was added to *pH*~8. The precipitate was filtered, washed many times with water and dried.

2.2.2. 2-Benzyl-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (2)

Yield - 73%; Mp. 259-260°C (lit. 265-268°C [21]), white crystals, recrystallized from ethanol; IR (KBr) ν(cm^{-1}): 3157 (NH), 2921 (CH), 1656 (Amide I); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.74-1.87 (m, 4H, $2CH_2$), 2.72 (t, *J* = 6.1 Hz, 2H, CH_2), 2.95 (t, *J* = 6.1 Hz, 2H, CH_2), 3.97 (s, 2H, CH_2), 7.21 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.25 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.34 (d, *J* = 7.3 Hz, 2H, Ar-H), 11.26 (s, 1H, NH, exchangeable with D_2O). Analysis: $C_{17}H_{16}N_2OS$, C, 68.89; H, 5.44; N, 9.45; O, 5.40; S, 10.82; Found: C, 68.91; H, 5.42; N, 9.47; O, 5.43; S, 10.80.

2.2.3. Ethyl 2-(4-oxo-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-2-yl)acetate (3)

Yield - 57%. Mp. 180-182°C (lit. 184-186°C [22]), white crystals, recrystallized from ethanol; IR (KBr) ν(cm^{-1}): 3080 (NH), 2840, 2860 (CH), 1750 (C=O), 1680 (Amide I), 1440, 1370 (defCH), 1080 (C-O); ^{1}H NMR (DMSO) δ(ppm): 1.16 (bt, 3H, CH_3), 1.72 (s, 4H, CH_2), 2.55 (bt, 4H, $2CH_2$), 2.80 (bt, 4H, $2CH_2$), 3.67 (s, 2H, CH_2CO), 4.08 (m, 2H, $2OC_2H_5$), 12.36 (s, 1H, NH, exchangeable with D_2O). Analysis: Calc. for: $C_{14}H_{16}N_2O_3S$: C, 57.52; H, 5.52; N, 9.58; O, 16.42; S, 10.97; Found: C, 57.54; H, 5.51; N, 9.60; O, 16.44; S, 10.95.

2.2.4. 2-(Pyridin-3-yl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (4)

Yield - 74% Mp. 289-290°C (lit. 302-305°C [3]), white crystals, recrystallized from dioxane; IR (KBr) ν(cm^{-1}): 3114 (NH), 2937 (CH), 1647 (Amide I); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.73-1.86 (m, 4H, $2CH_2$), 2.76 (t, *J* = 5.9 Hz, 2H, CH_2), 2.90 (t, *J* = 5.9 Hz, 2H, CH_2), 7.56 (dd, *J* = 8.0, 4.8 Hz, 1H, ArH), 8.40-8.44 (m, 1H, ArH), 8.72 (dd, *J* = 4.8, 1.5 Hz, 1H, ArH), 9.20 (d, *J* = 1.9 Hz, 1H, ArH), 12.72 (s, 1H, NH, exchangeable with D_2O). Analysis: calc. for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83; O, 5.65; S, 11.32; Found: C, 63.55; H, 4.64; N, 14.85; O, 5.62; S, 11.30.

2.2.5. 2-(Morpholinomethyl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (5)

Yield: 57%; Mp. 220-222°C, white crystals, recrystallized from ethyl acetate; IR (KBr) ν(cm^{-1}): 3159 (NH), 2946 (CH), 1655 (Amide I), 1449 (defCH), 1115 (C–O); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.74-1.84 (m, 4H, $2CH_2$), 2.47–2.55 (m, 4H, CH_2), 2.70 (dd, *J* = 8.3, 3.9 Hz, 2H, CH_2), 2.94 (dd, *J* = 8.3, 4.0 Hz, 2H, CH_2), 3.48 (s, 2H, CH_2), 3.66–3.76 (m, 4H, CH_2), 9.87 (s, 1H, NH, exchangeable with D_2O). Analysis: calc. for $C_{15}H_{19}N_3O_2S$; C, 58.99; H, 6.27; N, 13.76; O, 10.48; S, 10.50; Found: C, 58.02; H, 6.25; N, 13.74; O, 10.50; S, 10.47.

2.2.6. 2-(3,4,5-Trimethoxyphenyl)-5,6,7,8-tetrahydrothieno [2,3-d]pyrimidin-4(3H)-one (6)

Yield - 66%, Mp. 273-275°C, white crystals, recrystallized with ethanol [23]; IR (KBr) ν(cm^{-1}): 3179, 3122 (NH), 2935 (CH), 1655 (Amide I), 1125 (C–O); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.87–1.73 (m, 4H, $2CH_2$), 2.73 (t, *J* = 6.0 Hz, 2H, CH_2), 2.90 (t, *J* = 5.2 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.92 (s, 6H, $2OCH_3$), 7.27 (s, 2H, ArH), 11.42 (s, 1H, NH, exchangeable with D_2O); Analysis: Calc. for $C_{19}H_{20}N_2O_4S$, C, 61.27; H, 5.41; N, 7.52; O, 17.18; S, 8.61; Found: C, 61.25; H, 5.43; N, 7.54; O, 17.16; S, 8.64.

2.2.7. 2-(Pyridin-4-yl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (7)

Yield - 93% Mp. 318-320°C yellow crystals, recrystallized from dioxane, (lit. 311-314°C [3]); IR (KBr) ν(cm^{-1}): 3403, 3299 (NH), 2937 (CH), 1652 (Amide I); ^{1}H NMR (TFA/CH_3COOH) δ(ppm): 1.80–1.91 (m, 4H, $2CH_2$), 2.84 (t, *J* = 5.8 Hz, 2H, CH_2), 3.00 (t, *J* = 5.8 Hz, 2H, CH_2), 8.78 (d, *J* = 6.0 Hz, 2H, ArH), 9.04 (d, *J* = 6.1 Hz, 2H, ArH), 11.84 (s, 1H, NH, exchangeable with D_2O). Analysis: calc. for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83; O, 5.65; S, 11.32; Found: C, 63.55; H, 4.64; N, 14.85; O, 5.62; S, 11.33.

2.2.8. (2-(Pyridin-2-yl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (8)

Yield - 70%; Mp. 195-197°C, yellow crystals, recrystallized from dioxane (lit. 196-200°C [3]); IR (KBr) ν(cm^{-1}): 3308 (NH), 2936 (CH), 1676 (Amide I); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.85-1.96 (m, 4H, $2CH_2$), 2.81–2.87 (m, 2H, CH_2), 3.07–3.12 (m, 2H, CH_2), 7.47 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H, ArH), 7.90 (td, *J* = 7.7, 1.7 Hz, 1H, ArH), 8.46 (dt, *J* = 8.0, 1.0 Hz, 1H, ArH), 8.67 (ddd, *J* = 4.8, 1.6, 0.9 Hz, 1H, ArH), 10.88 (s, 1H, NH, exchangeable with D_2O). Analysis: calc. for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83; O, 5.65; S, 11.32; Found: C, 63.60; H, 4.64; N, 14.80; O, 5.62; S, 11.35.

2.2.9. 2-(2-Chloroethyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-d]pyrimidin-4(3H)-one (9)

Yield - 85%; Mp. 221–222 °C, white crystals, recrystallized from *i*-BuOH, (lit. 300–302°C [24]); IR (KBr) ν(cm^{-1}): 3304, 3250 (NH), 2940, 2867 (CH), 1666 (Amide I); ^{1}H NMR (DMSO) δ(ppm): 1.83–1.71 (m, 4H, $2CH_2$), 2.72 (t, *J* = 6.0 Hz, 2H, CH_2), 2.85 (t, *J* = 6.1 Hz, 2H, CH_2), 3.08 (t, *J* = 6.6 Hz, 2H, CH_2), 3.99 (t, *J* = 6.6 Hz, 2H, CH_2), 12.35 (s, 1H, NH, exchangeable with D_2O). Analysis: Calc. for

$C_{11}H_{11}ClN_2OS$: C, 51.86; H, 4.35; Cl, 13.92; N, 11.00; O, 6.28; S, 12.59.

2.2.10. 2-Methyl-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (10)

Yield - 92%; Mp. 306-309°C (lit. 302-305°C [25]); IR (KBr) ν(cm^{-1}): 3155 (NH), 2922, 2865, 2839 (CH), 1662 (Amide I); ^{1}H NMR (DMSO) δ(ppm): 1.85 (m, 4H, $2CH_2$), 2.33 (s, 3H, CH_3), 2.66 (t, 2H, CH_2), 2.83 (t, 2H, CH_2), 12.23 (bs, 1H, NH, exchangeable with D_2O); Calc. for $C_{11}H_{12}N_2OS$: C, 59.97; H, 5.49; N, 12.72; O, 7.26; S, 14.56; Found: C, 59.94; H, 5.51; N, 12.70; O, 7.23; S, 14.58.

2.2.11. 2-Ethyl-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (11)

Yield - 47%; Mp. 260-262°C, white crystals, recrystallized from *i*-PrOH (lit. 263-265°C [26]); IR (KBr) ν(cm^{-1}): 3405, 3298 (NH), 2936, 2840 (CH), 1662 (Amide I); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.41 (t, J = 7.6 Hz, 3H, CH_3), 1.84–1.94 (m, 4H, $2CH_2$), 2.74–2.85 (m, 4H, $2CH_2$), 3.03 (t, J = 6.0 Hz, 2H, CH_2), 12.17 (s, 1H, NH, exchangeable with D_2O); Calc. for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; O, 6.83; S, 13.68; Found: C, 61.54; H, 6.05; N, 11.93; O, 6.80; S, 13.64.

2.2.12. 2-(4-Nitrobenzyl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (12)

Yield - 64%; Mp. 195-197°C, white crystals, recrystallized from *i*-BuOH; IR (KBr) ν(cm^{-1}): 3408, 3200 (NH), 2942, 2877, 2834 (CH), 1655 (Amide I), 1520, 1346 (N-O); ^{1}H NMR (DMSO) δ(ppm): 1.66-1.85 (m, 4H, $2CH_2$), 2.70 (d, J = 6.1 Hz, 2H, CH_2), 2.84 (d, J = 6.1 Hz, 2H, CH_2), 4.09 (s, 2H, CH_2), 7.59 (d, J = 8.8 Hz, 2H, ArH), 8.15–8.23 (m, 2H, ArH), 12.55 (s, 1H, NH, exchangeable with D_2O).

2.2.13. 2-(3-Chlorobenzyl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (13)

Yield - 93%; Mp. 294–297°C, white crystals, no further purification was needed; IR (KBr) ν(cm^{-1}): 3005 (NH), 2927, 2861 (CH), 1657 (Amide I); ^{1}H NMR (DMSO) δ(ppm): 1.68-1.82 (m, 4H, $2CH_2$), 2.70 (d, J = 6.1 Hz, 2H, CH_2), 2.84 (t, J = 6.1 Hz, 2H, CH_2), 3.93 (s, 2H, CH_2), 7.28 (d, J = 7.4 Hz, 1H, ArH), 7.33 (ddd, J = 11.0, 8.3, 4.8 Hz, 2H, ArH), 7.41 (s, 1H, ArH), 12.47 (s, 1H, NH, exchangeable with D_2O).

2.2.14. 2-(3-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (14)

Yield - 88%, Mp. 319-320°C, yellow crystals; no further purification was needed; IR (KBr) ν(cm^{-1}): 3403 (NH), 3107 (ArH), 2987, 2942 (CH), 1657 (Amide I); ^{1}H NMR (DMSO) δ(ppm): 1.80 (dt, J = 9.1, 5.0 Hz, 4H, $2CH_2$), 2.78 (t, J = 6.0 Hz, 2H, CH_2), 2.92 (t, J = 6.0 Hz, 2H, CH_2), 7.77 (t, J = 7.9 Hz, 1H, ArH), 7.94 (d, J = 7.8 Hz, 1H, ArH), 8.43 (d, J = 7.9 Hz, 1H, ArH), 8.48 (s, 1H, ArH), 12.78 (s, 1H, NH, exchangeable with D_2O). Analysis: Calc. for $C_{17}H_{13}F_3N_2OS$, C, 58.28; H, 3.74; F, 16.27; N, 8.00; O, 4.57; S, 9.15; Found: C, 58.31; H, 3.72; F, 16.29; N, 8.02; O, 4.54; S, 9.17.

2.2.15. Synthesis of 5,6,7,8-tetrahydrothieno[2,3-d]pyrimidin-4(3H)-one (15)

A mixture of compound **1** (0.01 mol) and formamide (10 mL) was refluxed for 3h. The reaction mixture was allowed to stay overnight at room temperature. The solid product was filtered and washed with water. Yield - 90%, Mp. 257–258°C, white solid, recrystallized with ethanol (lit. 259–261°C [25]); IR (KBr) ν(cm^{-1}): 3156 (NH), 2938, 2857, 2815 (CH), 1658 (Amide I); ^{1}H NMR (DMSO) δ(ppm): 1.70−1.83 (m, 4H, $2CH_2$), 2.73 (t, J = 6.1 Hz, 2H, CH_2), 2.86 (dd, J = 8.3, 4.0 Hz, 2H, CH_2), 7.99 (s, 1H, N=CH), 12.67 (s, 1H, NH, exchangeable with D_2O).

2.3. Pharmacology

The parasitological study *in vitro* was carried out as described in [27-30]. For the screening, encapsulated infective larvae of *Trichinella spiralis* were used, 100 specimens per 1 mL physiological solution, released in advance from the muscle capsules by digestion with acid pepsin solution. The compounds were dissolved in DMSO with concentration of 100 μg/mL and 250 μg/mL. The samples were incubated in ''humid'' chamber with thermostat at 37°C. The response of *Trichinella spiralis* was observed after 24 h and 48 h, respectively by means of electronic microscope *MBC-9*.

3. RESULTS AND DISCUSSION

The synthesis of the studied compounds was performed as outlined in Scheme **1**.

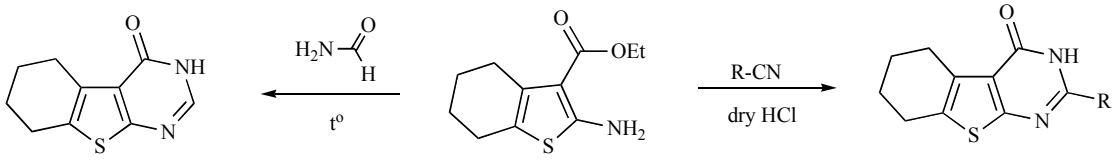
The physico-chemical properties of the synthesized 2-aminothiophene-3-carboxylate **1** corresponded to the data already published by Gewald [20].

The target compounds **2-14** were prepared by one-pot synthesis carried out as a cyclocondensation between 2-aminothiophene-3-carboxylate **1** and appropriate alkyl or aryl nitrile in the presence of dry dioxane by passing hydrogen chloride gas through the solution. This procedure led to the synthesis of 2-substituted thieno[2,3-*d*]pyrimidin-4-ones **2-14**. A different synthetic approach was used for the preparation of derivative compound **15** using the reaction procedure described in [25].

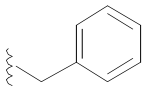
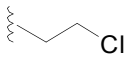
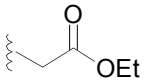
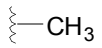
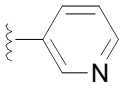
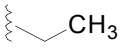
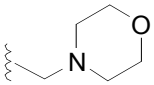
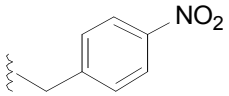
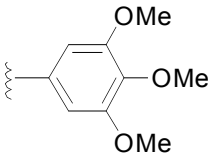
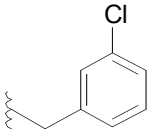
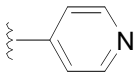
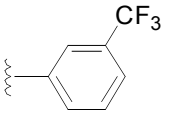
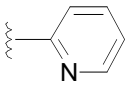
The 2-substituted-thieno[2,3-*d*]pyrimidin-4(3H)-ones **2-15** were obtained in good yields and seven of them were investigated for their cytotoxicity against encapsulated larvae of *Trichinella spiralis*.

The pharmacological test was performed with compounds **2, 4-8** and **15**, dissolved in DMSO. It was found as it can be seen from the data given in Table 1 that the tested compounds revealed different antihelminthic effects, expressed in suppressing the locomotor activity of *T. spiralis* larvae and loss of their spiral form, which is a sign of non-viability. In the control samples, both in physiological solution and in DMSO, practically all *T. spiralis* larvae were in spiral form, i.e., vital. Statistical significant differences in the level of larvae in both control and experimental groups were determined ($p \leq 0.05$).

The *in vitro* results obtained from the test showed that the most effective against *Trichinella* larvae is compound **2**. Its



15 **1** **2-14**

Compound	R	Compound	R
2		9	
3		10	
4		11	
5		12	
6		13	
7		14	
8		15	

Scheme 1. One-pot synthesis of thieno[2,3-*d*]pyrimidin-4(3H)-ones.

efficacy at concentration of 100 μg/mL and 250 μg/mL after 24 hours and after 48 hours reaches 97.94% and 100%, respectively. Compound **15** shows efficacy of 97.50 % after 48 hours at a concentration of 250 μg/mL. Furthermore, compounds **4** and **8** exhibit significant increase in the inhibitory effect at higher concentrations after 24 and 48 hours.

The data from the *in vitro* analysis showed that compounds **2**, **8** and **15** exhibit a higher activity than the reference drugs *albendazole* and *ivermectin* in the 24-hour incubation of samples at concentrations of 0.37, 0.35 and 0.48 μM resp. 0.92, 0.88 and 1.2 μM. Moderate efficacy was shown by thienopyrimidine **5** (59.90 %) and **6** (51.80 %) at a concentration of 0.33 and 0.27 μM relative to the reference drugs.

The observed antihelminthic effects of compounds **2, 4-8** and **15** show that introduction of benzyl substituent in the thieno[2,3-*d*]pyrimidin-4-one core is very beneficial to achieve high efficacy, while the presence of fragments containing more than one N- or O-atoms, as in compounds **5** and **6**, leads to moderate effects. Furthermore, on comparing the effects of compounds **4**, **7** and **8** it could be concluded that the presence of pyridin-4-yl substituent ensures higher activity than the presence of pyridin-3-yl or pyridin-2-yl fragment. The fact that all the tested thieno[2,3-*d*]pyrimidin-4-ones showed antihelminthic effects is a good evidence that the design of thienopyrimidines as purine and quinazolines bioisosters is a successful strategy for the development of new antiparasitic drugs.

The pharmacokinetic properties of the investigated thieno[2,3-*d*]pyrimidin-4-ones **2-15** have an essential role for the pharmacological potential *in vivo* i.e. sufficient bioavailability and transport across the membranes - to the binding site of the desired receptor as well as an optimal metabolism profile and elimination. The molecular properties such as lipophilicity, molecular size, flexibility and the presence of donors and proton acceptors can provide useful information in this connection (Table **2**). All reported data are computed using the *Molinspiration* software program [31].

Table 1. ***In vitro*** **activity against parasite larvae of** ***Trichinella spiralis*** **[a].**

Comp.	Efficacy (%)[b] After 24h		Efficacy (%)[b] After 48h		Concentrations 100 (250) μg/mL in μM
	100 μg/mL	250 μg/mL	100 μg/mL	250 μg/mL	
2	97.93	97.94	97.98	100.00	0.37 (0.92)
4	35.90	81.90	60.00	95.65	0.35 (0.88)
5	59.90	58.29	75.21	69.03	0.33 (0.82)
6	51.83	54.05	64.29	81.16	0.27 (0.67)
7	19.73	38.46	31.90	69.38	0.35 (0.88)
8	64.75	86.56	73.77	93.47	0.35 (0.88)
15	63.00	95.42	67.37	97.50	0.48 (1.20)
Albendazole	10.70	13.30 [c]	15.10	17.40 [c]	0.75
Ivermectin	48.60	54.30 [c]	78.60	88.20[c]	0.23

[a] Control – 96 parasites
[b] $p < 0.05$.
[c] Efficacy (%) at 200 μg/mL

Table 2. Calculated molecular properties of the tested compounds: Partition coefficient (logP), Molecular Weight (MW) [g/mol], Topologic Polar Surface Area (TPSA) [Å^2], molecular volume (Vol) [Å^3], sum of O and N H-bond acceptors (N_{HA}), sum of OH and NH H-bond donors (N_{HD}) and absorption (%ABS) [%].

Comp.	*LogP*	*MW*	*TPSA*	*Vol*	N_{HA}	N_{HD}	*%ABS*
2	3.99	296.4	45.75	263.1	3	1	93.22
4	2.96	283.4	58.65	242.2	4	1	88.77
5	1.67	305.4	58.23	305.4	5	1	88.91
6	3.67	372.5	73.46	323.0	6	1	83.66
7	2.75	283.4	58.65	242.2	4	1	88.77
8	2.89	283.4	58.65	242.2	4	1	88.77
15	1.92	206.3	45.75	174.9	3	1	93.22
Albendazole	2.75	265.3	67.02	234.1	5	2	85.88

Most of the antiparasitic drugs can reach their "targets" by oral administration (*via* the blood stream and/or the gastrointestinal tract) or by diffusion [32]. The Topological Polar Surface (TPSA) is a good indicator of the intestinal absorption of biologically active substances, with better bioavailability occurring at TPSA ≤ 140 Å^2 [33]. This parameter is used to calculate the percentage of absorption (% ABS) using the following equation (1):

$$\%ABS = 109\text{-}0.345\times TPSA \quad (1)\ [34, 35]$$

From the structure-activity relationship (SAR) data presented in Table **1**, it is obvious that all tested compounds **2**, **4**-**8** and **15** meet the requirements of the Lipinski's rule [36] (Table **2**). Moreover, all compounds have values of TPSA lower than 140 Å^2. The calculated intestinal absorption of thieno [2,3-*d*]pyrimidines **2**, **4**-**8** and **15** varies from 88.77% to 93.22% and it is higher than %ABS of *albendazole* (85.88%).

The pharmacological activity of benzimidazoles, including *albendazole*, is based on their affinity for specific receptors located within the target parasite: Beta-tubulin, acetylcholine channels and glutamate-closed chloride channels, respectively. The pharmacokinetic behavior of the antihelminthic drug includes the duration of drug absorption, tissue distribution, metabolism and excretion, which determines the concentration of the antihelminthic drug. Furthermore, the anthelminthics must reach their specific receptor in the target parasite to exercise their action. Therefore, entry of the drug into the parasite is critical for the anthelmintic efficacy. On the other side, it was observed that the higher partition coefficient (logP) for antiparasitic agents (relative to *albendazole*) corresponds to a higher ac-

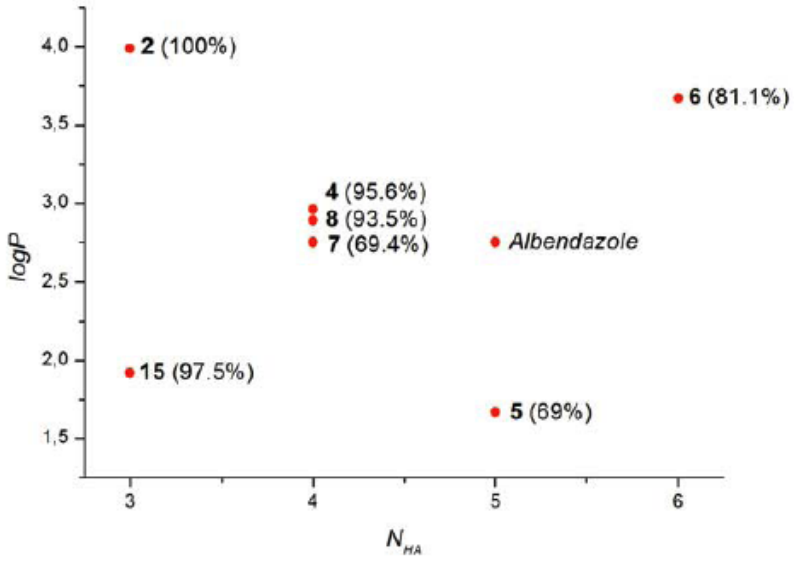


Fig. (2). SAR between N_{HA} and logP.

tivity. It was suggested that increased lipophilicity contributes to higher ability of biomechanical penetration [37]. Similar dependence is also observed for thienopyrimidines **2**, **4**-**8** and **15**, where increasing of logP and reduction of the number of H-bond acceptor groups (N_{HAD}) lead to higher antiparasitic activity (Fig. **2**). Considering the structural similarity between the synthesized compounds and the tyrosine kinase inhibitor, CRT 0064854, it can be assumed that the thienopyrimidine derivatives **7** and **8** exert their activity by inhibiting the secreted tyrosine kinases.

It was observed that compound **15** having the lowest logP value showed high antihelminthic activity. This was also observed with some *albendazole* derivatives, where those with the lowest logP (1.7) exhibited higher antiparasitic activity than *albendazole* against *T. spirallis* [38].

In order to obtain enough serum nutrients, the parasite increases its permeability to erythrocytes and a large set of small molecules through the aquaporins in the cell membrane [32, 39]. These changes in permeability are due to Plasmodial Surface Anion Channel (PSAC) [39].

Due to the fact that compound **15** has a low molecular volume (Vol) and logP, it can be supposed that the activity of the compound is due to a penetration through the ion channels of the parasitic cells, which explains the deviation of **15** from the rendered SAR model.

CONCLUSION

In summary, the *in vitro* screening for anthelminthic effect showed that thienopyrimidin-4-one derivatives exhibit higher anthelminthic effect than *albendazole* against *Trichinella spiralis*. Compounds **2**, **4**-**8** and **15** demonstrated higher absorption %ABS than *albendazole*. Thienopyrimidine **2** showed higher activity than *ivermectin* which is expressed as 100% non-viability of *Trichinella* larvae at dose of 0.92 µM after 48 h.

The SAR analysis showed that partition coefficient (logP) is essential for parasite drug penetration due to diffusion through the cell membrane. The activity increases as the molecule becomes more lipophilic. Some active compounds, such as thienopyrimidine **15,** probably penetrate through the ion channels of the parasitic cells due to their small molecular size.

The results of the *in vitro* screening for antiparasitic activity confirm the hypothesis that as bioisosters of the quinazolines, the thienopyrimidines exhibit an anti-trichinellosis activity.

DISCLOSURE

The authors confirm that the manuscript has not been published previously or under consideration for publication elsewhere.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest, including any financial, personal or other relationship with other people or institutions.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Lima, L.M.; Barreiro, E.J. Bioisosterism: A useful strategy for molecular modification and drug design. *Curr. Med. Chem*., **2005**, *12*(1), 23-49.

[2] Thornber, C.W. Isosterism and molecular modification in drug design. *Chem. Soc. Rev*., **1979**, *8,* 563-580.

[3] Haswani, N.G.; Bari, S.B. Synthesis and antimicrobial activity of novel 2-(pyridin-2-yl)thieno[2,3-d] pyrimidin-4(3H)-ones. *Turk J. Chem*., **2011**, *35*(6), 915-924.

[4] Cohen, A.; Suzanne, P.; Lancelot, J.-C.; Verhaeghe, P.; Lesnard, A.; Basmaciyan, L.; Hutter, S.; Laget, M; Dumètre, A.; Paloque,

L.; Deharo, E; Crozet, M.D.; Rathelot, P.; Dallemagne, P.; Lorthiois, A.; Sibley, C.H.; Vanelle, P.; Valentin, A.; Mazier, D.; Rault, S.; Azas, N. Discovery of new thienopyrimidinone derivatives displaying antimalarial properties toward both erythrocytic and hepatic stages of Plasmodium. *Eur. J. Med. Chem*., **2015**, *95*, 16-28.

[5] Hafez, H.N.; Al-Duaij, O.K.; El-Gazzar, A.-R.B.A. Design, synthesis and pharmacological evaluation of new nonsteroidal anti-inflammatory derived from 3-aminobenzothieno[2,3-d]pyrimidines. *Int. J. Org. Chem.*, **2013**, *3*(2), 110-118.

[6] Pédeboscq, S.; Gravier, D.; Casadebaig, F.; Hou, G.; Gissot, A.; Rey, C.; Ichas, F.; De Giorgi, F.; Lartigue, Pometan, L.J.-P. Synthesis and evaluation of apoptosis induction of thienopyrimidine compounds on KRAS and BRAF mutated colorectal cancer cell lines. *Biorg. Med. Chem*., **2012**, *20*(22), 6724-6731.

[7] Kotaiah, Y.; Harikrishna, N.; Nagaraju, K.; Rao, C.V. Synthesis and antioxidant activity of 1, 3, 4-oxadiazole tagged thieno[2,3-d] pyrimidine derivatives. *Eur. J. Med. Chem*., **2012**, *58*, 340-345.

[8] Modica, M.; Romeo, G.; Materia, L.; Russo, F.; Cagnotto, A.; Mennini, T.;. Gáspár, R.; Falkay, G.; Fülöp, F. Synthesis and binding properties of novel selective 5-HT 3 receptor ligands. *Biorg. Med. Chem*., **2004**, *12*(14), 3891-3901.

[9] Lawton, P. Purine analogues as antiparasitic agents. *Expert Opin. Ther. Pat.*, **2005**, *15*(8), 987-994.

[10] Berg, M.; Kohl, L.; Van der Veken, P.; Joossens, J.; Al-Salabi, M. I.; Castagna, V.; Giannese, F.; Cos, P.; Verse´es, W.; Steyaert, J.; Grellier, P.; Haemers, A.; Degano, M.; Maes, L.; de Koning, H. P.; Augustyns, K. Evaluation of nucleoside hydrolase inhibitors for treatment of *African Trypanosomiasis*. *Antimicrob. Agents Chemother*., **2010**, *54*(5): 1900-1908.

[11] Sarciron, M.E; Lawton, P.; Petavy, A.F.; Peyron, F. Alterations of *Toxoplasma gondii* induced by 2′, 3′-dideoxyinosine *in vitro*. *J. Parasitol*., **1998**, *84*(5), 1055-1059.

[12] Lawton, P.; Hejl, C.; Mancassola, R.; Naciri, M.; Petavy, A.-F. Effects of purine nucleosides on the *in vitro* growth of *Cryptosporidium parvum*. *FEMS Microbiol. Lett*., **2003**, *226*(1), 39-43.

[13] Woodring, J.L.; Patel, G.; Erath, J.; Behera, R.; Lee, P.J.; Leed, S. E.; Rodriguez, A.; Sciotti, R.J.; Mensa-Wilmot, K.; Pollastri, M.P. Evaluation of aromatic 6-substituted thienopyrimidines as scaffolds against parasites that cause trypanosomiasis, leishmaniasis, and malaria. *Med. Chem. Comm*., **2015**, *6*(2), 339-346.

[14] Dzik, J.M. Molecules released by helminth parasites involved in host colonization. *Acta Biochim. Pol*., **2006**, *53* (1), 33-64.

[15] Gounaris K. Nucleotidase cascades are catalyzed by secreted proteins of the parasitic nematode *Trichinella spiralis*. *Infect. Immun*., **2002**, *70*, 4917- 4924.

[16] Gounaris, K.; Selkirk, M.E. Parasite nucleotide-metabolizing enzymes and host purinergic signalling. *Trends Parasitol*., **2005**, *21*, 17-21.

[17] Gounaris, K.; Selkirk, M.E.; Sadeghi, S.J. A nucleotidase with unique catalytic properties is secreted by *Trichinella spiralis*. *Mol. Biochem. Parasitol*., **2004**, *136*(2), 257-264.

[18] Despommier, D.D. How does *Trichinella spiralis* make itself at home? *Parasitol. Today*, **1998**, *14*(8), 318-323.

[19] Kjær, S.; Linch, M.; Purkiss, A.; Kostelecky, B.; Knowles, P.P.; Rosse, C.; Riou, P.; Soudy, C.; Kaye, S.; Patel, B. Adenosine-binding motif mimicry and cellular effects of a thieno[2, 3-d]pyrimidine-based chemical inhibitor of atypical protein kinase C isoenzymes. *Biochem. J*., **2013**, *451*(2), 329-342.

[20] Gewald, K.; Schinke, E.; Böttcher, H. Heterocyclen aus CH-aciden Nitrilen, VIII. 2-Amino-thiophene aus methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel. *Chem. Ber*., **1966**, *99*(1), 94-100.

[21] Sánchez, A.I.; Meneses, R.; Mínguez, J.M.; Núñez, A.; Castillo, R.R.; Filace, F.; Burgos, C.; Vaquero, J.J.; Álvarez-Builla, J.; Cortés-Cabrera, A. Microwave-assisted synthesis of potent PDE7 inhibitors containing a thienopyrimidin-4-amine scaffold. *Org. Biomol. Chem*., **2014**, *12*(24), 4233-4242.

[22] Mavrova, A.T.; Wesselinova, D.; Tsenov, J.A.; Lubenov, L.A. Synthesis and antiproliferative activity of some new thieno [2, 3-d] pyrimidin-4(3H)-ones containing 1,2,4-triazole and 1,3,4-thiadiazole moiety. *Eur. J. Med. Chem*., **2014**, *86*, 676-683.

[23] Haggam, R.A.; Moustafa, A.H. Síntesis y actividad antimicrobiana de una nueva serie de tienopirimidinas condensados. *Afinidad*, **2014**, *71*(565), 68-73.

[24] Mavrova, A.T.; Vuchev, D.; Anichina, K.; Vassilev, N. Synthesis, antitrichinnellosis and antiprotozoal activity of some novel thieno[2, 3-d]pyrimidin-4 (3H)-ones containing benzimidazole ring. *Eur. J. Med. Chem*., **2010**, *45*(12), 5856-5861.

[25] Sauter, F. Beiträge zur Chemie schwefelhaltiger Heterocyclen, 1. Mitt.: Synthesen von Benzo[b]thieno[2,3-d]pyrimidin-Derivaten. *Monatsh. Chem*., **1968**, *99*(4), 1507-1514.

[26] Davoodnia, A.; Bakavoli, M.; Barakouhi, G.; Tavakoli-Hoseini, N. A new route to the synthesis of thieno [2,3-d] pyrimidin-4(3H)-one derivatives catalyzed by 12-tungstophosphoric acid (H3PW12O40). *Chin. Chem. Lett*., **2007**, *18*(12), 1483-1486.

[27] Despommier, D.D.; Campbell, W.C.; Blair, L. The *in vivo* and *in vitro* analysis of immunity to *Trichinella spiralis* in mice and rats. *Parasitol*., **1977**, *74*, 109-119.

[28] Arden, S.R.; Smith, A.M.; Booth, M.J.; Tweedie, S.; Gounaris, K.S.; Murray E. Identification of serine: Threonine protein kinases secreted by *Trichinella spiralis* infective larvae. *Mol. Biochem. Parasitol*., **1997**, *90*, 111-119.

[29] Mavrova, A.T.; Anichina, K.K.; Vuchev, D.I.; Tsenov, J.A.; Kondeva M.S.; Micheva, M.K. Synthesis and antitrichinellosis activity of some 2-substituted-[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-ones. *Bioor. Med. Chem*., **2005**, *13*, 5550-5559.

[30] Mavrova, A.T.; Anichina, K.K,; Vuchev, D.I.; Tsenov, J.A.; Denkova, P.S.; Kondeva, M.S.; Micheva, M.K. Antihelminthic activity of some newly synthesized 5(6)-(un)substituted-1H-benzimidazol-2-ylthioacetylpiperazine derivatives. *Eur. J. Med. Chem*., **2006**, *41*, 1412-1420.

[31] *Molinspiration Cheminformatics*, Molinspiration property engine v2016.10; www.molinspiration.com: **2017**.

[32] Mottier, L.; Alvarez, L.; Ceballos, L.; Lanusse, C. Drug transport mechanisms in helminth parasites: Passive diffusion of benzimidazole anthelmintics. *Exp. Parasitol*., **2006**, *113*(1), 49-57.

[33] Veber, D.F.; Johnson, S.R.; Cheng, H.-Y.; Smith, B.R.; Ward, K.W.; Kopple, K.D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem*., **2002**, *45*(12), 2615-2623.

[34] Barea, C.; Pabón, A.; Castillo, D.; Zimic, M.; Quiliano, M.; Galiano, S.; Pérez-Silanes, S.; Monge, A.; Deharo, E.; Aldana, I. New salicylamide and sulfonamide derivatives of quinoxaline 1,4-di-N-oxide with antileishmanial and antimalarial activities. *Biorg. Med. Chem. Lett*., **2011,** *21*(15), 4498-4502.

[35] Zhao, Y. H.; Abraham M. H.; Le, J.; Hersey, A.; Luscombe, C.N.; Beck, G.; Sherborne, B.; Cooper, I. Rate-limited steps of human oral absorption and QSAR studies. *Pharm. Res*., **2002**, *19*(10), 1446-1457.

[36] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev*., **2001**, *46*, 3-26.

[37] Márquez-Navarro, A.; Nogueda-Torres, B.; Hernández-Campos, A.; Soria-Arteche, O.; Castillo, R.; Rodríguez-Morales, S.; Yépez-Mulia, L.; Hernández-Luis, F. Anthelmintic activity of benzimidazole derivatives against *Toxocara canis* second-stage larvae and Hymenolepis nana adults. *Acta Trop*., **2009**, *109*(3), 232-235.

[38] Rivera, J.C.; Yépez-Mulia, L.; Hernández-Campos, A.; Moreno-Esparza, R.; Castillo, R.; Navarrete-Vázquez, G.; Fuentes-Noriega, I.; Jung-Cook, H. Biopharmaceutic evaluation of novel anthelmintic (1H-benzimidazol-5 (6)-yl) carboxamide derivatives. *Int. J. Pharm*., **2007**, *343*(1) 159-165.

[39] Bokhari, A. A.; Solomon, T.; Desai, S.A. Two distinct mechanisms of transport through the plasmodial surface anion channel, *J. Membr. Biol.*, **2008**, *226*(1-3), 27-34.