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Design, facile synthesis and anthelmintic activity of new O-substituted 6-methoxybenzothiazole-2-carbamates. Part II†

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In the framework of pursuing the design and synthesis of a new series of substituted 6-methoxybenzothiazole-2-carbamates as potential anthelmintics, and as a continuation of the expended efforts in part I, we have set out to develop novel compounds with enhanced anthelmintic activity by blocking the 6-position of benzothiazole with side chains of different polarities. Guided by the findings in part I, and reporting the paramphistomicidal activity of oxadiazoline derivatives V and VI, we aimed to synthesize target benzothiazoles designed to comprise some planar heterocyclic ring systems, namely, 1,3,4-oxadiazoles and 1,2,4-triazoles, bearing a variety of hydrophobic and hydrophilic components. The synthesis of the desired compounds was primarily achieved by cyclization of 6-acetohydrazide, 1. The *in vitro* paramphistomicidal activity of all synthesized carbamates was evaluated. Four synthesized carbamates exhibited notable activity. Compound 24, methyl 6-[(5-(4-bromophenacysulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate, displayed an equipotent effect to the reference drug oxclozanide at a concentration of 80 µg mL⁻¹; compounds 9, 10 and 23 showed high orders of anthelmintic effect. A structural computational study on the polar nature and hydrophilic-lipophilic properties of the synthesized carbamates was undertaken to discuss their structure-activity relationship (SAR). Besides, pharmacophore mapping was performed using eight active compounds as a training set. The generated pharmacophore model revealed five common features and was validated using fenbendazole, triclabendazole and triclabendazole sulfoxide.

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1. Introduction

Helminthiasis is a widespread infection that causes serious complications, and is one of the most serious public health problems worldwide.¹ The emergence of drug resistance, in parallel with recent deficiencies in launching new anthelmintic agents ready for clinical use, have caused a dire need for the development of new chemotherapeutic agents.² Benzothiazole-2-carbamates have been proven to possess pertinent anthelmintic activity that is almost comparable to benzimidazole isosteres.³ Both share a common mechanism for inhibiting the formation of microtubules through binding with β-tubulin monomers and preventing their polymerization.² This leads to a cascade of biochemical and physiological alterations, resulting in the loss of cellular homeostasis, disequilibrium

and finally parasite death.⁴ The mentioned pharmacophoric anthelmintics, having out-of-plane substituents, are more active than those with substituents in-plane, which proved, according to the theory of McCracken *et al.*, a highly polar,

Fig. 1 Reported benzothiazole-2-carbamates and 2-acetamido derivatives showing anthelmintic activity.

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L-shaped cleft to which drugs bind at hypothetical receptor active sites.⁴ Earlier literature reported the significant anthelmintic activity of *N*-[(2,6-dimethyl)- and *N*-[(2-ethyl-6-methyl)-phenyl]benzothiazol-2-ylsulfanyl-acetamide, **I** and **II** respectively (Fig. 1), where a 2-carbamoyl methylthio function acts as a spacer between two non-coplanar aromatic centers.^{5,6}

Related analogs, methyl 6-[5-(4-methylphenyl)amino-[1,3,4]-oxadiazol-2-yl]benzothiazole-2-carbamate, **III**, and methyl 6-(5-mercapto-4-phenyl-4*H*-[1,2,4]-triazol-3-yl)benzothiazol-2-carbamate, **IV**, showed promisingly high *in vitro* anthelmintic activity, causing 100% worm mortality at a dose of 45 $\mu\text{g mL}^{-1}$ after 2 and 4 hours exposure, respectively.³

Recently, in our preceding publication, part I, the anthelmintic effect of some newly designed and synthesized 6-methoxybenzothiazole derivatives as potentially active and metabolically stable compounds against inactivation by 6-hydroxylation and conjugation reactions was reported. Two of these latter synthesized compounds, namely, *N*-[6-[(4-acetyl-5-(4-chlorophenyl) and (4-nitrophenyl)-4,5-dihydro-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazol-2-yl]acetamides (**V** and **VI**) (Fig. 1), exhibited significant activity against rumen flukes. The presence of an almost rigid 1,3,4-oxadiazoline ring on the 6-methoxybenzothiazole skeleton was more favorable for paramephistomicidal activity, compared to flexible aliphatic substitutions. Molecular electron density distribution as well as the hydrophilic-lipophilic pattern of the oxadiazoline ring in compounds **V** and **VI** is possibly responsible for biomembrane transportation and anthelmintic activity.⁷ Consequently, guided by these findings, attention has increased toward the design and synthesis of hybrid 6-methoxybenzothiazole series combining two pharmacophores: a non derivatized 2-methylcarbamate and rigid non-coplanar ring systems, specifically 1,3,4-oxadiazoles or 1,2,4-triazoles linked to diverse sulfanyl functions (Fig. 2). Substituents were chosen so as to provide diverse functionalities with hydrogen bond donor, acceptor, charge transfer, π -stacking, hydrophobic and hydrophilic interacting groups. Computational studies of the influence of the polar nature and hydrophilic-lipophilic properties was undertaken. Interest in the anthelmintic activity of these analogs has initiated the structure-activity relationship (SAR) evaluation of different series. Designed molecules, containing both hydrophobic and hydrophilic components, should be interesting candidates for interacting with speculative receptor ligand binding sites.

Fig. 2 Potential design of 6-substituted benzothiazolecarbamate prototype anthelmintics.

2. Results and discussion

2.1. Chemistry

The target 6-methoxybenzothiazole derivatives were synthesized as revealed in the reactions depicted in Schemes 1 and 2. In Scheme 1, the starting material 2-(2-aminobenzothiazol-6-yloxy)acetohydrazide **1** was prepared, as reported earlier,⁷ by heating ethyl 2-(2-aminobenzothiazol-6-yloxy)acetate with hydrazine hydrate. Acid hydrazide **1** was refluxed with CS_2 and KOH in ethanol⁸ to yield oxadiazolethiole derivative **2a**, which tautomerized to thione **2b** (Fig. 3), as elucidated in its $^1\text{H-NMR}$ spectrum showing 2 D_2O -exchangeable singlets for highly deshielded *SH* at very low field, corresponding to structure **2a**, and for the oxadiazoline proton structure **2b**, at high field, each integrated for half proton, in addition to the usual signals for methylene protons and D_2O -exchangeable $\text{C}^2\text{-NH}_2$. 5-Substituted sulfanyl-[1,3,4]-oxadiazolyl derivatives **3–6** were prepared in better yields, following a procedure for the regio-selective alkylation of hydrazones by stirring a mixture of 1,3,4-oxadiazole-5-thiol derivative **2** and an equimolar amount of selected alkyl or aralkyl halide in $\text{K}_2\text{CO}_3/\text{DMF}$.⁹ The corresponding designed methyl 6-[(5-(alkyl- or aralkylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamates **7–10** were synthesized by stirring a mixture of substituted sulfanyl derivatives **3–6** and methyl chloroformate, adopting a previously reported procedure.⁷ Mass spectra of compounds **4** and **8** did not show molecular ion peaks. However, they displayed peaks at m/z (%) 279 (5) and 305 (0.4) corresponding to $\text{C}_{10}\text{H}_7\text{N}_4\text{O}_2\text{S}_2$, due to cleavage of the ethyl group, and to $\text{C}_{12}\text{H}_9\text{N}_4\text{O}_4\text{S}$, due to cleavage of the *S*-ethyl group. There was a base peak at m/z 223, corresponding to methyl 6-oxybenzothiazole-2-carbamate. Treatment of **1** with appropriately substituted aryl isothiocyanates led to direct cyclization into the corresponding triazole derivatives **11–13**. This was supposed to proceed through the formation of a non-isolated thiosemicarbazide (**a**) which tautomerizes into (**b**), followed by the spontaneous elimination of a molecule of H_2O (Fig. 4). IR spectra for **11–13** showed stretching bands due to NH_2 , $\text{C}=\text{N}$, as well as *SH*, indicating that products existed in imino-thiol form, **11a–13a**, rather than amino-thione form **11b–13b**. Attempts to separate thiosemicarbazide (**a**) by reacting **1** with appropriately substituted aryl isothiocyanates under various reaction conditions,^{10–13} led to direct cyclization into corresponding triazole derivatives **11–13**. The latter were correspondingly carbamoylated using methyl chloroformate producing target methyl 6-[(5-sulfanyl-4-(4-substituted phenyl)-[1,2,4]-triazol-3-yl)methoxy]benzothiazole-2-carbamates **14–16** in good yield.

Scheme 2 deals with the synthesis of new thioacetate, thio-phenylethanone, and chloroethanethioate derivatives as versatile 6-methoxybenzothiazole congeners. 2-Amino-6-[[5-ethoxycarbonylmethylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (**17**) was attained in very good yields by stirring the oxadiazole thiol derivative **2** with ethyl chloroacetate.⁹ Carbamoylation of **17** was performed by adopting the formerly depicted procedure to afford target carbamate **18**. Treatment of **2** with equimolar amounts of the appropriate

Scheme 1 Synthesis of target compounds **7–10** and **14–16**. Reagents and conditions: (i) CS_2 , KOH, EtOH reflux, 24 h, 96%; (ii) R_1X , K_2CO_3 , DMF, rt, 2 h, 45–95%; (iii) ClCOOCH_3 , pyridine, rt, 24 h, 54–94%; (iv) ArNCS , DMF, rt, 5 h, 66–79%; (v) ClCOOCH_3 , pyridine, rt, 24 h, 67–86%.

Scheme 2 Synthesis of target compounds **18**, **22–24** and **26**. Reagents and conditions: (i) $\text{ClCH}_2\text{COOEt}$, K_2CO_3 , dry DMF, rt, 1 h, 75%; (ii) ClCOOCH_3 , pyridine, rt, 24 h, 82%; (iii) $4\text{-RC}_6\text{H}_4\text{COCH}_2\text{Br}$, K_2CO_3 , DMF, rt, 1 h, 79–88%; (iv) ClCOOCH_3 , pyridine, rt, 24 h, 74–93%; (v) ClCH_2COCl , K_2CO_3 , rt, 2 h, 75%; (vi) ClCOOCH_3 , pyridine, rt, 24 h, 89%.

phenacyl bromide in $\text{K}_2\text{CO}_3/\text{DMF}$ provided 2-amino-6-[(5-(4-substituted phenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazoles (**19–21**), which were further reacted with methyl chloroformate to achieve the intended methyl 6-[(5-(4-substituted phenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamates (**22–24**). Acylation of sulfanyl 1,3,4-oxadiazole **2** into 2-amino-6-[(5-(chloroacetylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (**25**) was accomplished

by reaction with equimolar amounts of chloroacetyl chloride in $\text{K}_2\text{CO}_3/\text{dry DMF}$, in accordance with the general *S*-acylation procedure.⁹ The target methyl 6-[(5-(chloroacetylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate (**26**) was prepared by reacting **25** with methyl chloroformate in pyridine. Compounds were identified, and their chemical structures were confirmed by IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, DEPT and mass spectra, depicting appropriate absorption bands, chemical shifts and molecular ion peaks.

Fig. 3 Tautomeric interconversion of oxadiazolethione **2a** to oxadiazolinethione **2b**.

2.2. Biological evaluation

All synthesized carbamates were evaluated *in vitro* for their anthelmintic activity against rumen flukes (*paramphistomum*).

Fig. 4 Synthesis of triazole derivatives **11–13** through the formation of tautomeric thiosemicarbazides.

Different concentrations and time intervals were applied. Percentage mortality was calculated and compared to the reference drug oxylozanide (Table 1, Fig. 5). The screened carbamates shared an oxymethylene spacer separating the benzothiazole nucleus from the sulfanyl substituted heterocyclic 5-membered ring. It was generally obvious that higher concentrations of active compounds exhibited higher anthelmintic activity. As perceived from this preliminary anthelmintic screening result, three candidates were proven to have high activity against *paramphistomum*. The highest paramphistomicidal activity was displayed by compound **24**, with a *p*-bromophenacylsulfanyloxadiazolyl moiety attached through an oxymethylene spacer at the 6-position of the benzothiazole ring. Its activity was comparable to the standard reference oxylozanide; at a concentration of $80 \mu\text{g mL}^{-1}$, it exhibited

Fig. 5 *In vitro* activity of synthesized 6-methoxybenzothiazole-2-carbamates at a concentration of $80 \mu\text{g mL}^{-1}$ in DMSO on isolated rumen flukes (*paramphistomum*).

60% and 100% mortality at 1 h and 2 h exposure respectively, versus 50% and 100% mortality for oxylozanide, reflecting correspondingly higher, and equal activities to the reference drug at the same time points. Concerning compound **9** with an allylsulfanyloxadiazolyl moiety and compound **23** containing a *p*-chlorophenacylsulfanyloxadiazolyl moiety at the 6-position of the benzothiazole ring, they showed a high order of paramphistomicidal activity. They were capable of producing a 100% mortality rate at a concentration of $80 \mu\text{g mL}^{-1}$ upon exposure to worms for 4 h. However, at 2 h exposure time, compounds **9** and **23** revealed 40% and 70% mortality rates, respectively, demonstrating lower activities than reference oxylozanide at the same time point. Regarding compound **10**, it was considerably active against rumen flukes, showing a percentage mortality of 100% upon exposure to rumen flukes for 6 h at a concentration of $80 \mu\text{g mL}^{-1}$. At a shorter time of exposure, compound **10**, having a benzylsulfanyloxadiazolyl moiety, achieved percentage mortality of 90% and 60% at exposure times of 4 and 2 h, respectively, at the same concentration. At a lower concentration of $40 \mu\text{g mL}^{-1}$, the compounds showed percentage mortality less than the reference drug oxylozanide, except for two compounds, **10** and **23**, which displayed 30% and 40% mortality, respectively, at 1 h exposure, while the reference drug displayed 30% at the same time point. As far as carbamates **8**, **16**, **18** and **22** are concerned, they were less paramphistomicidal than the standard reference oxylozanide at low and high doses of $40 \mu\text{g mL}^{-1}$ and $80 \mu\text{g mL}^{-1}$, indicating that the nature of ring substitution on either the oxadiazole or triazole ring affected activity in a good way. A significant reduction in percentage mortality with low exposure times (60–50% and 80–60%) was observed for those compounds at 4 h and 6 h, respectively, at $80 \mu\text{g mL}^{-1}$ dose. Other compounds, in particular **7** and **26**, had low orders of activity of 40% and 50%, respectively, after 6 h of exposure at $80 \mu\text{g mL}^{-1}$. On the other hand, **14** and **15** failed to initiate any activity. The remarkable activity of halogenated compounds **23** and **24** may be attributed to the presence of electron withdrawing substituents on the *p*-position of the phenacyl group. The increased lipophilicity imparted by the halogen may increase tissue penetration and hence paramphistomicidal activity. In addition, the halogen

Table 1 *In vitro* activity of synthesized 6-methoxybenzothiazole-2-carbamates at different exposure times at concentrations of 40 and $80 \mu\text{g mL}^{-1}$ in DMSO on isolated rumen flukes (*paramphistomum*)

Compound	% mortality							
	$40 \mu\text{g mL}^{-1}$				$80 \mu\text{g mL}^{-1}$			
	1 h	2 h	4 h	6 h	1 h	2 h	4 h	6 h
7	10	10	20	20	10	20	30	40
8	10	40	50	80	20	60	60	80
9	20	30	50	70	20	40	100	
10	30	30	50	60	30	60	90	100
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	10	20	30	50	20	20	50	80
18	10	20	30	30	20	30	50	60
22	10	20	20	30	30	40	50	70
23	40	40	40	40	50	70	100	
24	20	40	60	60	60	100		
26	10	10	20	20	10	30	30	50
Oxylozanide	30	70	100		50	100		
DMSO	0	0	0	0	0	0	0	0

renders the *p*-position of the phenyl ring metabolically more stable, compared to the unsubstituted less lipophilic analogue 22. The latter might undergo metabolic oxidation to the *p*-hydroxyl derivative, demonstrating higher hydrophilic properties and less tissue penetration. An analogous approach may support the activity of *p*-chloro compound 16 with respect to inactive derivatives 14 and 15, which are lacking electron withdrawing halogens and are likely to undergo metabolic oxidation, *para*- and *p*-methyl hydroxylation respectively.

2.3. Molecular modeling studies

2.3.1. Molecular structure representation. Relying on structural computational studies on the polar nature and hydrophilic–lipophilic properties of synthesized carbamates, in correlation to their tested *in vitro* anthelmintic activity against the rumen fluke, the influence of different substituents at the 6-methoxybenzothiazole ring was reported. The assessment took into consideration two descriptors related to anthelmintic activity, cited in our previous work, part I,⁷ and detailed by McCracken *et al.*, concerning the percent polar surface area and molecular dipole, being related to biological transport and receptor binding.⁴ In the current work, molecular electronic differences were examined using a molecular modeling program. Electron densities were computationally calculated from molecular charges and hydrophilic–lipophilic regions. Colors were allocated according to electrostatic potential (EP, kcal mol^{−1}), ranging from regions of high electron density illustrated in red, to regions of low electron density illustrated in blue. The most promising compound was 24, with a *p*-bromophenacylsulfanyloxadiazolyl moiety attached through an oxymethylene spacer at the 6-position of the planar benzothiazole ring. Inspection of electronic and hydrophilic–lipophilic characteristics of 24, revealed that the oxadiazole ring created a new center for charge distribution, besides the methoxy oxygen and the carbamate moiety. They are the most negative parts of the molecule, drifting electrons from other parts, lowering the overall electron density on the benzothiazole ring, resulting in blue regions of less electron density at positions 2 and 5, and red regions of high electron density at positions 1, 3 and 4 of the oxadiazole ring. Also, the carbonyl group of the phenacyl moiety lowered the electron density on the phenacyl side chain to a considerable extent. In addition, the carbamate moiety lowered, to a lesser extent, the electron density of the benzothiazole ring (represented in green) (Fig. 6). It can also be assumed that the bromine atom at the 4-position of the phenacyl group imparted a high level of lipophilicity to the molecule (Fig. 7). Therefore, it was suggested that the overall polar surface area and lipophilicity imparted by 4-bromo substitution in compound 24 may be responsible for better transport properties and enhanced activity. Sequentially, a high order of paramphistomicidal activity was observed for two compounds: 9 with an allylsulfanyloxadiazolyl moiety, and 23 containing a *p*-chlorophenacylsulfanyloxadiazolyl moiety. Comparison of electronic and hydrophilic–lipophilic characteristics (Fig. 8

Fig. 6 Molecular structure representation of compound 24, colored based on molecular charges.‡

Fig. 7 Molecular structure representation of compound 24, colored based on lipophilicity.§

Fig. 8 Molecular structure representation of compound 24 (bottom drawing), compound 23 (middle drawing) and compound 9 (upper drawing), colored based on molecular charges.‡

and 9) of *p*-chlorophenacylsulfanyloxadiazolyl derivative 23 (middle drawing) to its sterically similar, yet slightly more active, *p*-bromophenacylsulfanyloxadiazolyl derivative 24 (bottom drawing), indicated that no obvious differences exist between the 2 molecules, except for halogen atoms, which indicates their essential role in lowering the electron density and increasing lipophilicity on the terminal side of the molecule. Similar conclusions can be drawn for allylsulfanyltriazolyl compound 9 (upper drawing) with a lesser influence of the allyl group. Comparing the electronic and hydrophilic–lipophilic characteristics of phenacylsulfanyloxadiazolyl

‡ Color codes for charges are as follows: blue = most positive, green = somewhat positive, white = neutral, yellow = somewhat negative and red = most negative.

§ Color codes by lipophilicity are as follows: blue = most lipophilic, turquoise = somewhat lipophilic, white = neutral, red = somewhat hydrophilic and dark red = most hydrophilic.

Fig. 9 Molecular structure representation of compound 24 (bottom drawing), compound 23 (middle drawing) and compound 9 (upper drawing), colored based on lipophilicity.‡

derivatives 22–24 showed no obvious differences among the 3 molecules (Fig. 10 and 11), except for the lack of electron withdrawing 4-substituents in compound 22 (upper drawing), being obviously less active. By withdrawing electrons from the phenacysulfanyl moiety, 4-bromo and 4-chloro substitutions create another center of high electronegativity (Fig. 10) and a region of hydrophobicity (Fig. 11) that could be responsible for binding to an active site on the receptor. Compound 22, lacking these elements, may not be competent for biologi-

Fig. 10 Molecular structure representation of compound 24 (bottom drawing), compound 23 (middle drawing) and compound 22 (upper drawing), colored based on molecular charges.‡

Fig. 11 Molecular structure representation of compound 24 (bottom drawing), compound 23 (middle drawing) and compound 22 (upper drawing), colored based on lipophilicity.§

cal transport or putative receptor fitting. Regarding benzylsulfanyloxadiazol 10, which is considerably active against rumen flukes with 100% percentage mortality for 6 h exposure at $80 \mu\text{g mL}^{-1}$, its electron densities and hydrophilic–lipophilic regions exhibited some similarities to the promising derivative 24 (Fig. 12 and 13), and their nearly entire superimposition was also evident (Fig. 14). Concerning the tested substituted triazolylcarbamates 14–16, having little or no anthelmintic activity, a comparison of their electronic, hydrophilic–lipophilic and steric properties with those of compound 24 (Fig. 15–17), indicated that a lack or reduction in activity for these derivatives 14–16 may be attributed to outlying interatomic distance differences, in contrast to compound 24, preventing the correct alignment and good fitting of these compounds into an active site on the receptor. In addition, there are some discrepancies in the electronic properties of compounds 14–16: charge densities around the

Fig. 12 Molecular structure representation of compound 10, colored based on molecular charges.‡

Fig. 13 Molecular structure representation of compound 10, colored based on lipophilicity.§

Fig. 14 Wire frame superimposition of compound 10 (red) and compound 24 (blue) molecules.

Fig. 15 Molecular structure representation of compound 24 (bottom middle drawing), compound 14 (right drawing) and compound 16 (left drawing), colored based on molecular charges.†

Fig. 16 Molecular structure representation of compound 24 (bottom middle drawing), compound 14 (right drawing) and compound 16 (left drawing), colored based on lipophilicity.‡

Fig. 17 Side view and wire frame superimposition of compound 16 (upper drawing, green): length = 16.2967, width = 9.01829, depth = 12.2323; compound 14 (middle drawing, pink): length = 16.2967, width = 9.01829, depth = 12.2323 and compound 24 (bottom drawing, violet): length = 21.8044, width = 10.3437, depth = 13.4195.

triazole ring are different, as indicated by the green (less positive) and white areas (neutral) in compound 14, instead of the blue (positive) and red areas (negative) in compound 24, accounting for differences in the percent polar surface area of the molecules.

2.3.2. Pharmacophore mapping. In this work pharmacophore mapping was performed using Molecular Operating Environment (MOE) 2008.10 (27).¹⁴ Compounds V, VI,⁷ 8–10, 16, 23, 24 were revealed as most active from preliminary anthelmintic screening against *paramphistomum*, and were chosen as the training set to generate a common feature pharmacophore model. The generated pharmacophore model consisted of five features (Fig. 18): one H-bond donor appearing as purple spherical mesh, one H-bond acceptor

Fig. 18 Generated pharmacophore hypothesis for anthelmintics. Pharmacophore features color codes: green = hydrophobic feature, purple = H-bond donor, turquoise = H-bond acceptor and orange = aromatic π system.

Fig. 19 Mapping of fenbendazole on selected pharmacophore hypothesis (rmsd = 0.808).

appearing as turquoise spherical mesh, two hydrophobic features appearing as green spherical meshes and one aromatic center feature appearing as an orange spherical mesh. This pharmacophore model showed accuracy of 100% and overlap of 7.4. Pharmacophore mapping depicted that the central benzothiazole ring was complementary, fitting a lipophilic group on the generated model. It was postulated that the 2-methyl carbamate fragment appeared to be essential for activity, where NH and carbonyl groups served as H-bond donor and H-bond acceptor, respectively. On the other hand, substitutions on the 6-position of the benzothiazole ring were thought to contribute through the hydrophobic oxymethylene spacer, where it might exist as a complementary lipophilic group. However, the rest of the chain at the 6-position of benzothiazole demonstrated no contribution, but might play a role in the proper alignment of active derivatives with their presumptive receptors. The generated pharmacophore model was validated using fenbendazole, triclabendazole and triclabendazole sulfoxide, showing potent anthelmintic activity against rumen flukes (*paramphistomum*)¹⁵ (Fig. 19–21). Mapping these compounds on the pharmacophore model demonstrated good fitting with root mean square deviation (rmsd) < 1 (Table 2).

Fig. 20 Mapping of triclabendazole sulfoxide on selected pharmacophore hypothesis (rmsd = 0.638).

Fig. 21 Mapping of triclabendazole on selected pharmacophore hypothesis (rmsd = 0.638).

Table 2 Structures and corresponding root mean square deviation (rmsd) of drugs used in the validation of the generated pharmacophore model

Validation compound	Structure	rmsd
Fenbendazole		0.808
Triclabendazole sulfoxide		0.638
Triclabendazole		0.638

3. Experimental section

3.1. Chemistry

Reagents and solvents were purchased from commercial suppliers and dried and purified when necessary, using standard techniques. Melting points were determined in open-glass capillaries using *Griffin* melting point apparatus and are all uncorrected. Infrared spectra (IR) were recorded, using KBr discs, ν (cm^{-1}), on a *Perkin-Elmer 1430* infrared spectrophotometer. Nuclear magnetic resonance (^1H -NMR and ^{13}C -NMR) were scanned on a *Mercury 300 MHz* spectrophotometer (Faculty of Science, Cairo University), *Jnm-Lambda series 400 MHz* spectrophotometer (Faculty of Science, Assiut University), and *Jeol NMR 500 MHz* spectrophotometer (Faculty of Science, Alexandria University). Chemical shifts are expressed as δ values (ppm) using tetramethylsilane (TMS) as the internal reference. DEPT (Distortionless Enhancement by Polarization Transfer) spectra were recorded on *Jnm-Lambda series 400 MHz* spectrophotometer (Faculty of Science, Assiut University). Mass spectra (MS) were run on a gas chromatograph/mass spectrometer *Shimadzu GCMS-Qp2010 plus* (70 eV), Faculty of Science, Cairo University. High resolution mass spectroscopy HRMS was performed on *Bruker Ultraflex 1MALDI TOF/TOF*, Friedrich Schiller University, Jena. Elemental microanalyses were performed at the microanalytical unit (Faculty of Pharmacy, Alexandria University) and at the microanalytical unit, Faculty of Science, Cairo University. Data were within $\pm 0.4\%$ of theoretical values. Reaction progress was monitored by thin layer chromatography (TLC) on silica gel (60 GF254, *Merck*) using glass plates, and spots were visualized by exposure to iodine vapors or to UV lamp at λ_{254} nm. Benzothiazoles 2–26 were synthesized by reactions depicted in Schemes 1 and 2.

3.1.1. 2-Amino-6-[(5-sulfanyl-[1,3,4]-oxadiazol-2-yl)-methoxy]benzothiazole (2). A suspension of the acid hydrazide 1 (0.71 g, 0.003 mole) and KOH (0.25 g, 0.0045 mole) in EtOH (25 mL) was treated with CS_2 (0.38 g, 0.005 mole). The reaction mixture was heated under reflux for 24 hours with continuous stirring, concentrated and the formed precipitate was dissolved in H_2O . The produced clear solution was acidified with 10% HCl to give a heavy white precipitate that was filtered, washed with H_2O , dried and recrystallized from EtOH/ H_2O (0.8 g, 96%); m.p.: 263 °C. IR ν (KBr, cm^{-1}): 3377, 3149 (NH_2), 2737 (SH), 1634 ($\text{C}=\text{N}$), 1595, 1477 ($\text{C}=\text{C}$ Ar), 1557, 1315, 1155 and 932 ($\text{N}-\text{C}=\text{S}$ amide), 1223, 1063 (ν_{as} and ν_{s} C–O–C). ^1H -NMR (DMSO- d_6 , 500 MHz) δ ppm: 4.00 (s, br, ^1H , oxadiazoline NH), 5.23 (s, 2H, OCH_2), 7.10 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.6$ Hz, $J_{\text{MX}} = 2.7$ Hz), 7.40 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 9.2$ Hz), 7.59 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 2.9$ Hz), 9.38 (s, br, 2H, $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 14.68 (s, ^1H , SH, D_2O -exchangeable). MS m/z (%): 280 (3), 279 (16), 167 (32), 150 (11), 149 (100), 113 (14), 112 (11), 83 (16), 71 (40), 70 (34), 69 (16), 57 (66), 56 (14), 55 (29). Anal. calcd for $\text{C}_{10}\text{H}_8\text{N}_4\text{O}_2\text{S}_2$: C, 42.85; H, 2.88; N, 19.99. Found: C, 42.56; H, 2.96; N, 19.88.

3.1.2. General procedure for the preparation of 2-amino-6-[(5-alkyl- or aralkylsulfanyl-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazoles (3–6). A stirred solution of 2 (0.56 g, 0.002 mole) and K_2CO_3 (0.1 g, 0.001 mole) in DMF (10 mL) was treated with the appropriate alkyl halide or aralkyl halide (0.002 moles). Stirring was continued at RT for 2 hours. The reaction mixture was then poured onto ice- H_2O while stirring, and the separated precipitates were filtered, washed with EtOH, dried and crystallized from EtOH. IR ν (KBr, cm^{-1}) of compounds 3–6: 3375–3348, 3111–3103 (NH_2), 1646–1635 ($\text{C}=\text{N}$), 1596–1542, 1472–1465 ($\text{C}=\text{C}$ Ar), 1294–1290, 1077–1055 (ν_{as} and ν_{s} C–S–C), 1268–1263, 1161–1115 (ν_{as} and ν_{s} C–O–C).

3.1.2.1. 2-Amino-6-[(5-methylsulfanyl-[1,3,4]-oxadiazol-2-yl)-methoxy]benzothiazoles (3). Yield: 54%, m.p.: 183–185 °C. ^1H -NMR (DMSO- d_6 , 300 MHz) δ ppm: 2.71 (s, 3H, S- CH_3), 5.32 (s, 2H, OCH_2), 6.92 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.8$ Hz, $J_{\text{MX}} = 2.4$ Hz), 7.25 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.7$ Hz), 7.28 (s, 2H, $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 7.43 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 2.4$ Hz). MS m/z (%): 294 (16), 165 (100), 137 (12), 130 (7), 122 (5), 110 (7), 83 (3), 75 (14), 69 (3), 59 (1). Anal. calcd for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}_2\text{S}_2$: C, 44.88; H, 3.42; N, 19.03; S, 21.79. Found: C, 44.96; H, 3.29; N, 18.68; S, 21.79.

3.1.2.2. 2-Amino-6-[(5-ethylsulfanyl-[1,3,4]-oxadiazol-2-yl)-methoxy]benzothiazoles (4). Yield: 95%, m.p.: 165–167 °C. ^1H -NMR (DMSO- d_6 , 500 MHz) δ ppm: 1.31 (t, 3H, S- CH_2CH_3 , $J = 7.6$ Hz), 3.25 (q, 2H, S- CH_2CH_3 , $J = 7.6$ Hz), 5.30 (s, 2H, OCH_2), 6.89 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 9.1$ Hz, $J_{\text{MX}} = 3$ Hz), 7.21 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 9.1$ Hz), 7.28 (s, 2H, $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 7.40 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 3$ Hz). MS m/z (%): 279 (5), 167 (16), 149 (47), 129 (13), 113 (11), 111 (13), 98 (13), 97 (25), 96 (13), 95 (17), 85 (24), 84 (19), 83 (38), 82 (17), 81 (33), 73 (31), 71 (55), 70 (28), 69 (68), 68 (13), 67 (21), 60 (34), 57 (100). Anal. calcd for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_2\text{S}_2$: C, 46.74; H, 3.92; N, 18.17; S, 20.80. Found: C, 47.11; H, 3.59; N, 18.56; S, 20.61.

3.1.2.3. 2-Amino-6-[(5-allylsulfanyl-[1,3,4]-oxadiazol-2-yl)-methoxy]benzothiazoles (5). Yield: 45%, m.p.: 110–112 °C. ^1H -

NMR (DMSO- d_6 , 300 MHz) δ ppm: 3.72 (d, 2H, S-CH₂, J = 7.2 Hz), 5.05–5.11 (m, 2H, allylC²-H), 5.37 (s, 2H, OCH₂), 5.74–5.86 (m, 1H, allylC¹-H), 6.95 (dd, 1H, C⁵-H, J_{AM} = 8.8 Hz, J_{MX} = 2.4 Hz), 7.32 (d, 1H, C⁴-H, J_{AM} = 8.7 Hz), 7.49 (s, 2H, C²-NH₂, D₂O-exchangeable), 7.62 (d, 1H, C⁷-H, J_{MX} = 2.4 Hz). Anal. calcd for C₁₃H₁₂N₄O₂S₂·2H₂O: C, 43.81; H, 4.52; N, 15.72; S, 17.99. Found: C, 43.67; H, 4.19; N, 15.86; S, 18.13.

3.1.2.4. 2-Amino-6-[(5-benzylsulfanyl)-[1,3,4]-oxadiazol-2-yl]-methoxy]benzothiazoles (6). Yield: 89%, m.p.: 181–183 °C. ¹H-NMR (DMSO- d_6 , 500 MHz) δ ppm: 4.45 (s, 2H, S-CH₂), 5.30 (s, 2H, OCH₂), 6.88 (dd, 1H, C⁵-H, J_{AM} = 8.4 Hz, J_{MX} = 2.2 Hz), 7.21 (d, 1H, C⁴-H, J_{AM} = 8.4 Hz), 7.25–7.43 (m, 6H, 5 Ar-Hs + C⁷-H), 7.29 (s, 2H, C²-NH₂, D₂O-exchangeable, overlapping with the multiplet assigned for Ar-Hs). Anal. calcd for C₁₇H₁₄N₄O₂S₂: C, 55.12; H, 3.81; N, 15.12; S, 17.31. Found: C, 54.90; H, 4.19; N, 14.84; S, 17.56.

3.1.3. General procedure for the preparation of methyl 6-[(5-alkyl- or aralkylsulfanyl)-[1,3,4]-oxadiazol-2-yl]methoxy]benzothiazole-2-carbamates (7–10). Methyl chloroformate (0.28 g, 0.003 mole) was added dropwise to a well stirred solution of amino compounds 3–6 (0.001 mole) in pyridine (7 L). The reaction mixture was stirred for 24 hours at RT and poured onto ice-H₂O to precipitate the products, which were filtered, washed thoroughly with H₂O, dried and recrystallized from DMF/H₂O. IR ν (KBr, cm⁻¹) of compounds 7–10: 3170–3084 (NH), 1731–1727 (C=O), 1611–1602 (C=N), 1592–1586, 1474–1470 (C=C Ar), 1272–1234, 1065–1054 (ν_{as} and ν_s C–O–C).

3.1.3.1. Methyl 6-[(5-(methylsulfanyl)-[1,3,4]-oxadiazol-2-yl) methoxy]benzothiazole-2-carbamate (7). Yield: 63%, m.p.: 235–237 °C. ¹H-NMR (DMSO- d_6 , 300 MHz) δ ppm: 2.71 (s, 3H, S-CH₃), 3.77 (s, 3H, NHCOOCH₃), 5.41 (s, 2H, OCH₂), 7.12 (d, 1H, C⁵-H, J_{AM} = 8.4 Hz), 7.61 (d, 1H, C⁴-H, J_{AM} = 8.7 Hz), 7.70 (s, 1H, C⁷-H), 11.96 (s, 1H, C²-NHCOOCH₃, D₂O-exchangeable). MS m/z (%): 352 (25), 320 (2), 305 (2), 263 (0.5), 238 (5), 237 (40), 223 (100), 207 (3), 191 (15), 165 (5), 138 (9), 129 (15), 83 (4), 80 (5), 59 (32). Anal. calcd for C₁₃H₁₂N₄O₄S₂: C, 44.31; H, 3.43; N, 15.90; S, 18.20. Found: C, 44.60; H, 3.81; N, 15.64; S, 18.01.

3.1.3.2. Methyl 6-[(5-(ethylsulfanyl)-[1,3,4]-oxadiazol-2-yl) methoxy]benzothiazole-2-carbamate (8). Yield: 54%, m.p.: 203–205 °C. ¹H-NMR (DMSO- d_6 , 500 MHz) δ ppm: 1.33 (t, 3H, S-CH₂CH₃, J = 7.6 Hz), 3.22 (q, 2H, S-CH₂CH₃, J = 7.6 Hz), 3.74 (s, 3H, NHCOOCH₃), 5.39 (s, 2H, OCH₂), 7.09 (d, dist, 1H, C⁵-H, J_{AM} = 8.4 Hz), 7.58 (d, 1H, C⁴-H, J_{AM} = 8.4 Hz), 7.67 (s, 1H, C⁷-H), 11.96 (s, 1H, C²-NHCOOCH₃, D₂O-exchangeable). MS m/z (%): 305 (0.4), 224 (13), 223 (100), 179 (11), 144 (12), 89 (10), 59 (19). Anal. calcd for C₁₄H₁₄N₄O₄S₂: C, 45.89; H, 3.85; N, 15.29; S, 17.50. Found: C, 46.30; H, 3.96; N, 15.16; S, 17.74.

3.1.3.3. Methyl 6-[(5-(allylsulfanyl)-[1,3,4]-oxadiazol-2-yl) methoxy]benzothiazole-2-carbamate (9). Yield: 75%, m.p.: 191–192 °C. ¹H-NMR (DMSO- d_6 , 300 MHz) δ ppm: 3.72 (d, 2H, S-CH₂, J = 7.2 Hz), 3.77 (s, 3H, NHCOOCH₃), 5.05–5.11 (m, 2H, allylC²-H), 5.37 (s, 2H, OCH₂), 5.74–5.86 (m, 1H, allylC¹-H), 6.95 (dd, 1H, C⁵-H, J_{AM} = 8.8 Hz, J_{MX} = 2.4 Hz), 7.32 (d, 1H, C⁴-H, J_{AM} = 8.7 Hz), 7.49 (s, 2H, C²-NH₂, D₂O-exchangeable), 7.62 (d, 1H, C⁷-H, J_{MX} = 2.4 Hz), 11.96 (s, 1H, C²-NHCOOCH₃, D₂O-exchangeable). MS m/z (%): 378 (16), 224 (14), 223 (100), 191 (18), 179 (15), 151

(11), 138 (12), 122 (17), 109 (16), 95 (12), 92 (11), 83 (12), 79 (72), 78 (69), 77 (29), 76 (39), 75 (35), 74 (22), 71 (13), 69 (20). Anal. calcd for C₁₅H₁₄N₄O₄S₂·2H₂O: C, 43.47; H, 4.38; N, 13.52; S, 15.47. Found: C, 43.16; H, 3.99; N, 13.21; S, 15.68.

3.1.3.4. Methyl 6-[(5-(benzylsulfanyl)-[1,3,4]-oxadiazol-2-yl) methoxy]benzothiazole-2-carbamate (10). Yield: 94%, m.p.: 205–207 °C. ¹H-NMR (DMSO- d_6 , 500 MHz) δ ppm: 3.74 (s, 3H, NHCOOCH₃), 4.47 (s, 2H, S-CH₂), 5.38 (s, 2H, OCH₂), 7.08 (d, 1H, C⁵-H, J_{AM} = 8.4 Hz), 7.23–7.37 (m, 5H, 5 Ar-Hs), 7.58 (d, 1H, C⁴-H, J_{AM} = 8.7 Hz), 7.66 (s, 1H, C⁷-H), 11.97 (s, 1H, C²-NHCOOCH₃, D₂O-exchangeable). MS m/z (%): 428 (12), 397 (6), 237 (16), 223 (100), 207 (7), 206 (18), 192 (14), 178 (22), 161 (30), 124 (45), 123 (39), 97 (18), 58 (36). Anal. calcd for C₁₉H₁₆N₄O₄S₂: C, 53.26; H, 3.76; N, 13.08; S, 14.97. Found: C, 53.18; H, 3.74; N, 12.86; S, 14.72.

3.1.4. General procedure for the preparation of 2-amino-6-[[4-(4-substituted phenyl)-5-sulfanyl-[1,2,4]-triazol-3-yl] methoxy]benzothiazoles (11–13). A suspension of the acid hydrazide 1 (0.95 g, 0.004 mole) in DMF (20 mL) was treated with the appropriate selected aryl isothiocyanate (0.0044 mole). The reaction mixture was stirred at RT for 5 hours where the starting material gradually dissolved on progress of reaction. The final clear reaction mixture was poured onto ice-H₂O to precipitate products which were filtered, dried, washed with diethyl ether and recrystallized from EtOH/H₂O. IR ν (KBr, cm⁻¹) of compounds 11–13: 3432–3406, 3307–3203 (NH₂), 2720–2651 (SH), 1642–1618 (C=N), 1581–1524, 1493–1467 (C=C Ar), 1227–1209, 1019–1015 (ν_{as} and ν_s C–O–C).

3.1.4.1. 2-Amino-6-[[4-(4-phenyl-5-sulfanyl-[1,2,4]-triazol-3-yl) methoxy]benzothiazole (11). Yield: 66%, m.p.: 255–257 °C. ¹H-NMR (DMSO- d_6 , 500 MHz) δ ppm: 4.96 (s, 2H, OCH₂), 6.84 (dd, 1H, C⁵-H, J_{AM} = 8.4 Hz, J_{MX} = 2.5 Hz), 7.30 (d, 1H, C⁴-H, J_{AM} = 8.4 Hz), 7.36 (d, 1H, C⁷-H, J_{MX} = 2.3 Hz), 7.42–7.51 (m, 5H, Ar-H), 9.32 (s, br, 2H, C²-NH₂, D₂O-exchangeable), 14.04 (s, 1H, SH, D₂O-exchangeable). MS m/z (%): 355 (23), 190 (18), 166 (100), 165 (46), 137 (8), 122 (3), 82 (1), 77 (22), 69 (5), 55 (13). Anal. calcd for C₁₆H₁₃N₅OS₂· $\frac{1}{2}$ H₂O: C, 52.73; H, 3.87; N, 19.22; S, 17.60. Found: C, 52.93; H, 3.85; N, 18.98; S, 17.73.

3.1.4.2. 2-Amino-6-[[4-(4-methylphenyl)-5-sulfanyl-[1,2,4]-triazol-3-yl) methoxy]benzothiazole (12). Yield: 75%, m.p.: 200–202 °C. ¹H-NMR (DMSO- d_6 , 500 MHz) δ ppm: 2.32 (s, 3H, *p*-tolyl CH₃), 4.86 (s, 2H, OCH₂), 6.66 (dd, 1H, resolved after deuteration, C⁵-H, J_{AM} = 8.6 Hz, J_{MX} = 2.3 Hz), 7.07 (d, 1H, C⁷-H, J_{MX} = 2.3 Hz), 7.14 (d, 1H, C⁴-H, J_{AM} = 8.4 Hz), 7.21 (d, 2H, C^{3',5'}-Hs, J = 7.6 Hz, overlapping with C²-NH₂, D₂O-exchangeable), 7.26 (d, 2H, C^{2',6'}-Hs, J = 7.6 Hz), 13.99 (s, 1H, SH, D₂O-exchangeable). ¹³C-DEPT-135 (DMSO- d_6 , 400 MHz) δ ppm: 20.74 (CH₃), 60.96 (OCH₂), 107.62 (C-4), 114.78 (C-5), 116.97 (C-7), 127.78 (Ar-C-3,5), 129.72 (Ar-C-2,6). MS m/z (%): 369 (15), 205 (15), 204 (17), 167 (12), 166 (100), 165 (52), 145 (9), 137 (17), 110 (18), 91 (41), 77 (16), 69 (22), 65 (30), 56 (10), 55 (39), 52 (26), 51 (24). HRMS m/z (%): 370.451 (100) (M^+ + 1, C₁₇H₁₅N₅OS₂). Anal. calcd for C₁₇H₁₅N₅OS₂: C, 55.26; H, 4.09; N, 18.96; S, 17.36. Found: C, 54.98; H, 3.92; N, 18.91; S, 17.23.

3.1.4.3. 2-Amino-6-[[4-(4-chlorophenyl)-5-sulfanyl-[1,2,4]-triazol-3-yl) methoxy]benzothiazole (13). Yield: 79%, m.p.: 293–

295 °C. $^1\text{H-NMR}$ (DMSO-d_6 , 500 MHz) δ ppm: 4.92 (s, 2H, OCH_2), 6.67 (d, dist, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.15 (d, dist, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.7$ Hz), 7.18 (s, 1H, $\text{C}^7\text{-H}$), 7.48 (d, 2H, $\text{C}^{2',6'}\text{-Hs}$, $J = 6.9$ Hz), 7.57 (d, 2H, $\text{C}^{3',5'}\text{-Hs}$, $J = 6.9$ Hz, overlapping with $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 14.07 (s, 1H, SH , D_2O -exchangeable). MS m/z (%): 391 (7), 389 (15), 224 (10), 167 (15), 166 (94), 165 (100), 138 (17), 137 (23), 111 (20), 110 (21), 80 (10), 75 (15), 69 (14). Anal. calcd for $\text{C}_{16}\text{H}_{12}\text{ClN}_5\text{OS}_2\cdot\text{C}_2\text{H}_5\text{OH}$: C, 49.59; H, 4.16; N, 16.06; S, 14.71. Found: C, 49.62; H, 4.09; N, 16.44; S, 14.60.

3.1.5. General procedure for the preparation of methyl 6-[(4-(4-substituted phenyl)-5-sulfanyl-[1,2,4]-triazol-3-yl)-methoxy]benzothiazole-2-carbamates (14–16). Methyl chloroformate (0.28 g, 0.003 mole) was dropwise added to a stirred solution of compounds 11–13 (0.0011 mole) in pyridine (7 mL) and the mixture was stirred for 24 hours at RT. It was then poured onto ice- H_2O to precipitate products which were filtered, washed thoroughly with H_2O , dried and crystallized from DMF/ H_2O . IR ν (KBr, cm^{-1}) of compounds 14–16: 3166–3081 (NH), 1728–1727 (C=O), 1616–1613 (C=N), 1568–1559, 1498–1469 (C=C Ar), 1247–1215, 1055 (ν_{as} and ν_{s} C–O–C).

3.1.5.1. Methyl 6-[(4-phenyl-5-sulfanyl-[1,2,4]-triazol-3-yl)-methoxy]benzothiazole-2-carbamate (14). Yield: 85%, m.p.: 232–234 °C. $^1\text{H-NMR}$ (DMSO-d_6 , 500 MHz) δ ppm: 3.73 (s, 3H, NHCOOCH_3), 4.96 (s, 2H, OCH_2), 6.82 (dd, resolved after deuteration, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.4$ Hz, $J_{\text{MX}} = 2.5$ Hz), 7.44 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.1$ Hz), 7.46 (s, 1H, $\text{C}^7\text{-H}$), 7.48–7.50 (m, 5H, Ar-Hs), 11.93 (s, 1H, NHCOOCH_3 , D_2O -exchangeable), 14.03 (s, 1H, SH , D_2O -exchangeable). $^{13}\text{C-NMR}$ (DMSO-d_6 , 300 MHz) δ ppm: 52.93 (CH_3), 60.94 (OCH_2), 106.81 (C-4), 115.26 (C-5), 120.77 (C-7), 128.09 ($ph\text{-C-3} + ph\text{-C-5}$), 129.27, 129.37, 129.52 ($ph\text{-C-2} + ph\text{-C-6} + ph\text{-C-4}$), 132.43 ($ph\text{-C-1}$), 133.44 (C-7a), 144.18 (C-3a), 148.02 (C-6), 153.63 (C-2), 154.45 (Triaz-C-3), 158.10 (Triaz-C-5), 168.63 (C=O). MS m/z (%): 354 (22), 339 (4), 206 (100), 192 (8), 176 (10), 166 (21), 165 (78), 164 (56), 132 (36), 130 (27), 113 (29), 91 (12), 76 (84), 74 (21). Anal. calcd for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{O}_3\text{S}_2$: C, 52.29; H, 3.66; N, 16.94; S, 15.51. Found: C, 52.38; H, 4.01; N, 16.76; S, 15.59.

3.1.5.2. Methyl 6-[(4-(4-methylphenyl)-5-sulfanyl-[1,2,4]-triazol-3-yl)methoxy]benzothiazole-2-carbamate (15). Yield: 67%, m.p.: 228–230 °C. $^1\text{H-NMR}$ (DMSO-d_6 , 500 MHz) δ ppm: 2.30 (s, 3H, $p\text{-tolyl CH}_3$), 3.73 (s, 3H, NHCOOCH_3), 4.94 (s, 2H, OCH_2), 6.85 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.9$ Hz, $J_{\text{MX}} = 1.5$ Hz), 7.25–7.35 (m, 4H, Ar-Hs, resolved into 2d, after deuteration, each integrated for 2H at 7.23 for $\text{C}^{3',5'}\text{-Hs}$, $J = 8.4$ Hz and at 7.26 for $\text{C}^{2',6'}\text{-Hs}$, $J = 8.4$ Hz), 7.42 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 1.5$ Hz), 7.49 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 11.94 (s, 1H, NHCOOCH_3 , D_2O -exchangeable), 13.99 (s, 1H, SH , D_2O -exchangeable). MS m/z (%): 339 (1), 219 (40), 218 (91), 204 (22), 191 (12), 167 (19), 166 (70), 165 (50), 145 (17), 139 (19), 138 (24), 137 (20), 131 (21), 118 (10), 112 (22), 110 (26), 105 (19), 95 (13), 91 (100), 89 (13). Anal. calcd for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_3\text{S}_2$: C, 53.38; H, 4.01; N, 16.38; S, 15.00. Found: C, 53.56; H, 4.14; N, 16.55; S, 14.88.

3.1.5.3. Methyl 6-[(4-(4-chlorophenyl)-5-sulfanyl-[1,2,4]-triazol-3-yl)methoxy]benzothiazole-2-carbamate (16). Yield: 86%, m.p.: 242–244 °C. $^1\text{H-NMR}$ (DMSO-d_6 , 500 MHz) δ ppm: 3.73 (s, 3H, NHCOOCH_3), 4.99 (s, 2H, OCH_2), 6.84 (dd, 1H,

$\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.8$ Hz, $J_{\text{MX}} = 2.3$ Hz), 7.43 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 2.3$ Hz), 7.47 (d, overlapping with d of $\text{C}^{2',6'}\text{-Hs}$, resolved after deuteration, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.49 (d, 2H, $\text{C}^{2',6'}\text{-Hs}$, $J = 8.4$ Hz), 7.56 (d, 2H, $\text{C}^{3',5'}\text{-Hs}$, $J = 8.4$ Hz), 11.93 (s, 1H, NHCOOCH_3 , D_2O -exchangeable), 14.05 (s, 1H, SH , D_2O -exchangeable). MS m/z (%): 403 (16), 402 (11), 240 (40), 239 (31), 237 (19), 224 (18), 193 (14), 192 (21), 168 (14), 167 (32), 166 (100), 165 (81), 164 (21), 112 (54), 111 (41), 73 (29), 71 (25), 70 (37), 68 (30), 67 (21), 62 (19), 60 (52). Anal. calcd for $\text{C}_{18}\text{H}_{14}\text{ClN}_5\text{O}_3\text{S}_2$: C, 48.27; H, 3.15; N, 15.64; S, 14.32. Found: C, 48.59; H, 3.07; N, 15.47; S, 14.54.

3.1.6. 2-Amino-6-[[5-ethoxycarbonylmethylsulfanyl]-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (17). A mixture of 2 (0.56 g, 0.002 mole) and anhydrous K_2CO_3 (0.1 g, 0.001 mole) in dry DMF (10 mL), was treated with the equimolar amount of ethyl chloroacetate. The reaction mixture was stirred at RT for 1 hour and diluted with H_2O . The separated product was filtered, washed with H_2O and recrystallized from EtOH as pale yellowish crystals (0.55 g, 75%); m.p.: 129–130 °C. IR ν (KBr, cm^{-1}): 3351–3103 (NH_2), 1713 (C=O), 1639 (C=N), 1542, 1465 (C=C Ar), 1291, 1066 (ν_{as} and ν_{s} C–O–C). MS m/z (%): 366 (8), 213 (14), 166 (15), 165 (85), 149 (10), 147 (10), 138 (11), 137 (16), 129 (17), 125 (11), 123 (12), 122 (11), 120 (11), 119 (15), 115 (12). Anal. calcd for $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_4\text{S}_2\cdot 2\text{H}_2\text{O}$: C, 41.78; H, 4.51; N, 13.92; S, 15.94. Found: C, 41.78; H, 4.29; N, 13.65; S, 15.68.

3.1.7. Methyl 6-[[5-ethoxycarbonylmethylsulfanyl]-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate (18). Methyl chloroformate (0.28 g, 0.003 mole) was added dropwise to a stirred solution of compounds 17 (0.4 g, 0.0011 mole) in pyridine (7 mL) and the reaction mixture was stirred for 24 hours at RT. The final reaction mixture was poured onto ice- H_2O to precipitate the product, which was filtered, washed thoroughly with H_2O , dried and recrystallized from EtOH as white crystals (0.38 g, 82%); m.p.: 180–182 °C. IR ν (KBr, cm^{-1}): 3111 (NH), 1737 (C–C=O), 1731 (N–C=O), 1622 (C=N), 1565, 1473 (C=C Ar), 1266, 1035 (ν_{as} and ν_{s} C–O–C). $^1\text{H-NMR}$ (DMSO-d_6 , 500 MHz) δ ppm: 1.13 (t, 3H, $\text{O-CH}_2\text{CH}_3$, $J = 6.5$ Hz), 3.74 (s, 3H, NHCOOCH_3), 4.08 (q, 2H, $\text{O-CH}_2\text{CH}_3$, $J = 6.9$ Hz), 4.12 (s, 2H, SCH_2), 5.39 (s, 2H, OCH_2), 7.08 (d, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.58 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.66 (s, 1H, $\text{C}^7\text{-H}$), 11.97 (s, 1H, NHCOOCH_3 , D_2O -exchangeable). MS m/z (%): 424 (15), 225 (6), 224 (12), 223 (100), 202 (11), 201 (9), 191 (15), 147 (8), 138 (7), 59 (20). Anal. calcd for $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_6\text{S}_2$: C, 45.28; H, 3.80; N, 13.20; S, 15.11. Found: C, 44.87; H, 3.89; N, 12.88; S, 15.32.

3.1.8. General procedure for preparation of 2-amino-6-[(5-(4-substituted phenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)-methoxy]benzothiazoles (19–21). A stirred solution of the mercaptoamine 2 (0.56 g, 0.002 mole) and K_2CO_3 (0.1 g, 0.001 mole) in DMF (10 mL) was treated with substituted phenacyl bromides (0.002 moles) and stirring was continued at RT for 1 hour. The reaction mixture was then poured onto ice- H_2O while stirring. The separated precipitates were filtered, washed with H_2O , dried and recrystallized from EtOH. IR ν (KBr, cm^{-1}) of compounds 19–21: 3436–3434, 3173–3168 (NH_2), 1727 (C=O), 1676–1644 (C=N), 1581–1554, 1475–1462 (C=C Ar), 1269–1222, 1081–1066 (ν_{as} and ν_{s} C–O–C).

3.1.8.1. 2-Amino-6-[(5-phenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (19). Yield: 79%, m.p.: 163–165 °C. ^{13}C -DEPT-135 (DMSO- d_6 , 400 MHz) δ ppm: 40.60 (SCH_2), 60.25 (OCH_2), 107.42 (C-4), 113.84 (C-5), 118.02 (C-7), 128.44 (Ar-C-3,5), 128.90 (Ar-C-2,6), 134.01 (Ar-C-4). MS m/z (%): 398 (9), 280 (1), 179 (3), 165 (100), 149 (5), 137 (13), 134 (2), 122 (12), 119 (2), 110 (11), 105 (62), 91 (20), 77 (53), 51 (30). Anal. calcd for $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_3\text{S}_2$: C, 54.26; H, 3.54; N, 14.06; S, 16.09. Found: C, 54.52; H, 3.43; N, 14.23; S, 15.86.

3.1.8.2. 2-Amino-6-[(5-(4-chlorophenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (20). Yield: 82%, m.p.: 190–191 °C. ^1H -NMR (DMSO- d_6 , 300 MHz) δ ppm: 5.10 (s, 2H, SCH_2), 5.32 (s, 2H, OCH_2), 6.90 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.8$ Hz, $J_{\text{MX}} = 2.4$ Hz), 7.23 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.8$ Hz), 7.28 (s, 2H, $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 7.40 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 2.4$ Hz), 7.63 (d, 2H, $\text{C}^{3',5'}\text{-Hs}$, $J = 8.4$ Hz), 8.03 (d, 2H, $\text{C}^{2',6'}\text{-Hs}$, $J = 8.1$ Hz). MS m/z (%): 434 (2), 432 (5), 270 (1), 268 (3), 165 (100), 141 (25), 139 (83), 137 (12), 129 (0.6), 122 (8), 113 (13), 111 (40), 87 (1), 85 (4), 83 (4), 76 (21), 69 (8), 63 (6), 51 (10). Anal. calcd for $\text{C}_{18}\text{H}_{13}\text{ClN}_4\text{O}_3\text{S}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$: C, 48.92; H, 3.19; N, 12.68; S, 14.51. Found: C, 49.22; H, 3.39; N, 12.35; S, 14.43.

3.1.8.3. 2-Amino-6-[(5-(4-bromophenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (21). Yield: 88%, m.p.: 187–189 °C. ^1H -NMR (DMSO- d_6 , 500 MHz) δ ppm: 5.08 (s, 2H, SCH_2), 5.29 (s, 2H, OCH_2), 6.87 (dd, dist, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.4$ Hz, $J_{\text{MX}} = 3.1$ Hz), 7.20 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.35 (s, 2H, $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 7.38 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 3.2$ Hz), 7.76 (d, 2H, $\text{C}^{3',5'}\text{-Hs}$, $J = 8.4$ Hz), 7.93 (d, 2H, $\text{C}^{2',6'}\text{-Hs}$, $J = 8.4$ Hz). MS m/z (%): 478 (3), 476 (3), 184 (23), 167 (7), 166 (21), 165 (100), 156 (11), 155 (10), 138 (9), 137 (13), 122 (12), 110 (11), 90 (8), 89 (8), 80 (7), 77 (9), 69 (9), 63 (13), 55 (10), 51 (11), 50 (20). Anal. calcd for $\text{C}_{18}\text{H}_{13}\text{BrN}_4\text{O}_3\text{S}_2$: C, 45.29; H, 2.74; N, 11.74; S, 13.43. Found: C, 45.41; H, 2.91; N, 11.58; S, 13.49.

3.1.9. General procedure for preparation of methyl 6-[(5-(4-substituted phenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamates (22–24). Methyl chloroformate (0.28 g, 0.003 mole) was added dropwise to a well stirred solution of compounds 19–21 (0.0011 mole) in pyridine (7 mL). The reaction mixture was stirred for 24 hours at RT and poured onto ice- H_2O . The precipitated products were filtered, washed thoroughly with H_2O , dried and recrystallized from EtOH. IR ν (KBr , cm^{-1}) of compounds 22–24: 3173–3170 (NH), 1738–1730 (C=O), 1727–1726 (N-C=O), 1676–1667 (C=N), 1586–1571, 1474–1471 (C=C Ar), 1274–1230, 1088–1066 (ν_{as} and ν_{s} C-O-C).

3.1.9.1. Methyl 6-[(5-phenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate (22). Yield: 74%, m.p.: 188–190 °C. ^1H -NMR (DMSO- d_6 , 300 MHz) δ ppm: 3.77 (s, 3H, NHCOOCH_3), 5.08 (s, 2H, SCH_2), 5.40 (s, 2H, OCH_2), 7.08–8.04 (m, 8H, $\text{C}^{4,7}\text{-Hs}$ + phenyl-Hs), 11.95 (s, 1H, NHCOOCH_3 , D_2O -exchangeable). MS m/z (%): 456 (4), 223 (38), 165 (11), 105 (100), 91 (20), 79 (15), 78 (11), 77 (80), 75 (16), 69 (12), 59 (24), 52 (19), 51 (47), 50 (22). Anal. calcd for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_5\text{S}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$: C, 51.60; H, 3.68; N, 12.04; S, 13.78. Found: C, 51.52; H, 3.45; N, 11.93; S, 13.85.

3.1.9.2. Methyl 6-[(5-(4-chlorophenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate (23). Yield: 93%, m.p.: 231–232 °C. ^1H -NMR (DMSO- d_6 , 300 MHz) δ ppm:

3.77 (s, 3H, NHCOOCH_3), 5.10 (s, 2H, SCH_2), 5.40 (s, 2H, OCH_2), 7.08 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 9.6$ Hz, $J_{\text{MX}} = 2.7$ Hz), 7.10–8.047 (m, 6H, $\text{C}^{4,7}\text{-Hs}$ + phenyl $\text{C}^{2',3',5',6'}\text{-Hs}$), 11.95 (s, 1H, NHCOOCH_3 , D_2O -exchangeable). MS m/z (%): 492 (1), 490 (3), 460 (1), 458 (2), 379 (1), 352 (2), 338 (1), 320 (2), 306 (1), 305 (0.7), 279 (2), 223 (34), 191 (13), 179 (4), 165 (13), 155 (1.5), 153 (2), 141 (31), 139 (100), 123 (34), 113 (5), 111 (46), 75 (28). Anal. calcd for $\text{C}_{20}\text{H}_{15}\text{ClN}_4\text{O}_5\text{S}_2 \cdot 2\text{H}_2\text{O}$: C, 45.58; H, 3.63; N, 10.63; S, 12.17. Found: C, 45.35; H, 3.57; N, 10.96; S, 12.21.

3.1.9.3. Methyl 6-[(5-(4-bromophenacylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate (24). Yield: 92%, m.p.: 228–230 °C. ^1H -NMR (DMSO- d_6 , 400 MHz) δ ppm: 3.76 (s, 3H, NHCOOCH_3), 5.09 (s, 2H, SCH_2), 5.40 (s, 2H, OCH_2), 7.08 (d, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.58 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.4$ Hz), 7.66 (s, 1H, $\text{C}^7\text{-H}$), 7.76 (d, 2H, $\text{C}^{3',5'}\text{-Hs}$, $J = 7.2$ Hz), 7.93 (d, 2H, $\text{C}^{2',6'}\text{-Hs}$, $J = 7.6$ Hz), 11.99 (s, 1H, NHCOOCH_3 , D_2O -exchangeable). MS m/z (%): 536 (4), 534 (4), 506 (3), 504 (3), 477 (6), 475 (7), 380 (3), 351 (1), 278 (3), 223 (22), 199 (11), 197 (12), 191 (22), 185 (96), 183 (100), 169 (37), 167 (40), 74 (14), 59 (21). Anal. calcd for $\text{C}_{20}\text{H}_{15}\text{BrN}_4\text{O}_5\text{S}_2$: C, 44.87; H, 2.82; N, 10.46; S, 11.98. Found: C, 44.48; H, 3.19; N, 10.26; S, 12.26.

3.1.10. 2-Amino-6-[(5-(chloroacetylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole (25). Chloroacetyl chloride (0.25 g, 0.0022 mole) was added dropwise to a stirred, ice-cooled solution of the sufanyloxadiazole 2 (0.56 g, 0.002 mole) and K_2CO_3 (0.1 g, 0.001 mole) in DMF (10 mL). Stirring was continued at RT for 2 hours and the reaction mixture was poured onto ice- H_2O , whereupon the separated product was filtered, washed with H_2O , dried and recrystallized from DMF/ H_2O as white crystals (0.54 g, 75%); m.p.: 179–180 °C. IR ν (KBr , cm^{-1}): 3345–3296 (NH_2), 1727 (C=O), 1640 (C=N), 1599, 1471 (C=C Ar), 1268, 1021 (C-S-C), 1219, 1166 (ν_{as} and ν_{s} C-O-C). ^1H -NMR (DMSO- d_6 , 300 MHz) δ ppm: 4.22 (s, 2H, ClCH_2), 5.33 (s, 2H, OCH_2), 6.91 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 8.5$ Hz, $J_{\text{MX}} = 2.7$ Hz), 7.24 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 8.7$ Hz), 7.28 (s, 2H, $\text{C}^2\text{-NH}_2$, D_2O -exchangeable), 7.42 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 2.7$ Hz). MS m/z (%): 358 (0.002), 356 (0.01), 202 (10), 167 (5), 166 (11), 165 (100), 138 (6), 137 (8), 122 (6), 110 (6), 100 (5). Anal. calcd for $\text{C}_{12}\text{H}_9\text{ClN}_4\text{O}_3\text{S}_2$: C, 40.39; H, 2.54; N, 15.70; S, 17.97. Found: C, 40.78; H, 2.87; N, 15.41; S, 17.89.

3.1.11. Methyl 6-[(5-(chloroacetylsulfanyl)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate (26). Methyl chloroformate (0.28 g, 0.003 mole) was added dropwise to a stirred solution of 25 (0.0011 mole) in pyridine (7 mL). The reaction mixture was stirred for 24 hours at RT and then poured onto ice- H_2O to precipitate the product, which was filtered, washed thoroughly with H_2O , dried and recrystallized from EtOH as pale yellow crystals (0.40 g, 89%); m.p.: 193–195 °C. IR ν (KBr , cm^{-1}): 3175 (NH), 1736 (C=O), 1730 (N-C=O), 1615 (C=N), 1543, 1473, (C=C Ar), 1292, 1162 (C-S-C), 1254, 1057 (ν_{as} and ν_{s} C-O-C). ^1H -NMR (DMSO- d_6 , 500 MHz) δ ppm: 3.73 (s, 3H, OCH_3), 4.188 (s, 2H, ClCH_2), 5.38 (s, 2H, OCH_2), 7.06 (dd, 1H, $\text{C}^5\text{-H}$, $J_{\text{AM}} = 9.2$ Hz, $J_{\text{MX}} = 2.3$ Hz), 7.56 (d, 1H, $\text{C}^4\text{-H}$, $J_{\text{AM}} = 9.2$ Hz), 7.65 (d, 1H, $\text{C}^7\text{-H}$, $J_{\text{MX}} = 2.3$ Hz), 12.04 (s, 1H, NH , D_2O -exchangeable). MS m/z (%): 417 (3), 415 (9), 223 (20), 164 (100), 148 (11), 137 (4), 122 (3). Anal. calcd for $\text{C}_{14}\text{H}_{11}\text{ClN}_4\text{O}_5\text{S}_2 \cdot \text{H}_2\text{O}$: C, 38.85; H, 3.03; N, 12.94; S, 14.82. Found: C, 38.63; H, 3.11; N, 12.92; S, 14.86.

3.2. Biological evaluation methodology

Adult rumen flukes, collected from animal rumen in a slaughter house, were added to a physiological solution prepared from 5.54 g NaCl, 0.35 g KCl, 0.28 CaCl₂, 2.1 g NaHCO₃, 0.29 g MgSO₄, 2.1 g glucose in distilled H₂O to 1 L and maintained at 37 °C. After ensuring the normal motility of worms, different concentrations of compounds under test in DMSO (40 µg mL⁻¹ and 80 µg mL⁻¹) were added and the motility of worms was monitored. Oxyclozanide 3.4% w/v suspension (Vetindia Pharmaceutical Limited, Hyderabad, India) was used as the reference drug, while DMSO was used as the control. The number of worms, affected by the presence of each test compound, was counted at different time intervals and the percentage mortality was calculated and compared to the paramphistomicidal activity of the reference drug¹⁶ (Table 1 and Fig. 5).

3.3. Molecular modeling studies

3.3.1. Molecular structural studies. Molecular structural representations, by molecular electronic differences and lipophilicity, were built using Sigma-Aldrich Molecular Modeling ProTM software. Colors are according to electrostatic potential (EP, kcal mol⁻¹), ranging from regions of high electron density illustrated in red, to regions of low electron density illustrated in blue.

3.3.2. Pharmacophore mapping. Compounds were built using the Builder module of the Molecular Operating Environment (MOE) 2008.10 (27),¹⁴ and subjected to energy minimization using MMFF94X force field. Pharmacophore model generation was achieved using the unified scheme present in the pharmacophore elucidation module. Among the generated pharmacophore models, the one with highest accuracy and overlap was selected for further investigation.

4. Conclusions

In this study, the notably high paramphistomicidal activity of four of our synthesized carbamates was affirmed. The most promising was methyl 6-[(5-(4-bromophenacylsulfany)-[1,3,4]-oxadiazol-2-yl)methoxy]benzothiazole-2-carbamate, **24**, which was equipotent to the reference drug oxyclozanide at a concentration of 80 µg mL⁻¹, showing percentage mortality of 60% and 100% at 1 h and 2 h exposure, respectively. Superior anthelmintic activity related to this compound, along with its favorable molecular representation features, led to the speculation that its improved potency could have been due to the contribution of specific functionalities notably comprising the phenacylsulfanyloxadiazolyl moiety attached through an oxymethylene spacer at the 6-position of the planar benzothiazole ring. Subsequent interest was directed towards compounds **9**, **10** and **23**, which shared matching electron densities and lipophilic patterns with **24**. Compounds deviating in molecular charges and hence polar surface area, and possessing different arrangements of molecular lipophilicity were obviously less active. This endorses the theory of McCracken *et al.*, relating drug anthelmintic activity to percent polar surface area and molecular dipole, regarding both transport and receptor binding.⁴ Furthermore, steric aspects of chemical groups and molecular

interatomic distances affected the structural alignment of new compounds and consequently, their activity. A successful pharmacophore model was generated from eight structures of our most active compounds, and was validated using fenbendazole, triclabendazole and triclabendazole sulfoxide, displaying good fitting with root mean square deviation (rmsd) less than 1. The authors believe that the introduction of the aforementioned groups to the benzothiazole core would provide insights into molecular accommodation and requirements for paramphistomicidal activity, and that compound **24** can be considered as a lead structure for the design of high potency benzothiazole anthelmintics in future studies. Finally, the present study may serve as the corner stone for the construction of new candidates, enabling the control of helminth infestation.

Conflict of interest

The authors declare no conflict of interest.

Notes and references

- 1 T. L. Lemke, in *Foye's Principles of Medicinal Chemistry*, ed. T. L. Lemke, A. D. Williams, V. F. Roche and W. S. Zito, Lippincott Williams and Wilkins, a Wolters Kluwer business, Philadelphia, USA, 7th edn, 2013, ch. 34, pp. 1125–1157.
- 2 R. K. Griffith, in *Burgers Medicinal Chemistry and Drug Discovery*, ed. D. J. Abraham, Wiley, J. and Sons, New York, USA, 6th edn, 2003, ch. 20, vol. 5, pp. 1089–1096.
- 3 A. M. M. E. Omar, I. C. Ahmed, O. A. El-Sayed, A. M. Youssef, D. A. Hassan and I. El-Ashrawy, *Alexandria J. Pharm. Sci.*, 2007, 21, 75–86.
- 4 R. O. McCracken and K. B. Lipkowitz, *J. Parasitol.*, 1990, 76, 853–864.
- 5 E. Sidoova, J. Danek and V. Konecny, *CS276322B6*, 1992.
- 6 E. Sidoova, J. Danek and V. Konecny, *CS276388B6*, 1992.
- 7 A. M. M. E. Omar, O. M. Aboulwafa, D. A. E. Issa, M. M. S. El-Shokrofy, M. E. Amr and I. M. El-Ashrawy, *MedChemComm*, 2015, 6, 795–805.
- 8 M. S. M. Abd alla, M. I. Hegab, N. A. Abo Taleb, S. M. Hasabelnaby and A. Goudah, *Eur. J. Med. Chem.*, 2010, 45, 1267–1277.
- 9 D. J. Brondani, D. R. D. M. Moreira, M. P. A. de Farias, F. R. D. S. Souza, F. F. Barbosa and A. C. L. Leite, *Tetrahedron Lett.*, 2007, 48, 3919–3923.
- 10 H. Bayrak, A. Demirbas, N. Demirbas and S. A. Karaoglu, *Eur. J. Med. Chem.*, 2010, 45, 4726–4732.
- 11 K. A. Mansour, M. M. Eid and S. N. Khalil, *Molecules*, 2003, 8, 744–755.
- 12 A. M. M. E. Omar and O. M. Aboulwafa, *J. Heterocycl. Chem.*, 1984, 21, 1419–1423.
- 13 A. M. M. E. Omar and N. H. Eshba, *J. Pharm. Sci.*, 1984, 73, 1166–1168.
- 14 *Molecular Operating Environment (MOE)*, C. C. Group, Montreal, Canada, 2008.10.27 edn, 2006.
- 15 P. F. Rolfe and J. C. Boray, *Aust. Vet. J.*, 1988, 65, 148–150.
- 16 D. J. Finney, *Probit Analysis*, Cambridge University Press, Cambridge, 2nd edn, 1962.