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# A linear discrimination analysis based virtual screening of trichomonacidal lead-like compounds: Outcomes of in silico studies supported by experimental results

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**Abstract**—A computational (virtual) screening test to identify potential trichomonacidals has been developed. Molecular structures of trichomonacidal and non-trichomonacidal drugs were represented using stochastic and non-stochastic atom-based quadratic indices and a linear discrimination analysis (LDA) was trained to classify molecules regarding their antiprotozoan activity. Validation tests revealed that our LDA-QSAR models recognize at least 88.24% of trichomonacidal lead-like compounds and suggest using this methodology in virtual screening protocols. These classification functions were then applied to find new lead antitrichomonal compounds. In this connection, the biological assays of eight compounds, selected by computational screening using the present models, give good results (87.50% of good classification). In general, most of the compounds showed high activity against *Trichomonas vaginalis* at the concentration of 100 μg/ml and low cytotoxicity to this concentration. In particular, two heterocyclic derivatives (VA7-67 and VA7-69) maintained their efficacy at 10 μg/ml with an important trichomonacidal activity (100.00% of reduction), but it is remarkable that the compound VA7-67 did not show cytotoxic effects in macrophage cultivations. This result opens a door to a virtual study considering a higher variability of the structural core already evaluated, as well as of other chemicals not included in this study.


Therefore, predictive modeling has the potential to transform early-stage drug discovery.

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Parasitic illnesses are among the most widespread of human diseases.[1] The infections due to protozoan parasites constitute the major health and economic problem, where Chagas' disease occupies the third place in number of deaths per year, after malaria and schistosomiasis.[2] On the other hand, trichomoniasis, an important sexually transmitted protozoa disease that is frequent in rich countries, also needs new effective agents.

The treatment of trichomoniasis was revolutionized by metronidazole (MTZ).[3] The introduction of this drug several decades ago heralded a new era in the treatment of infections caused by a range of pathogenic protozoan parasites.[3–7] In fact, MTZ is the drug now most widely used in the treatment of anaerobic protozoan parasitic infections caused by *Trichomonas vaginalis*, *Giardia duodenalis*, and *Entamoeba histolytica*.[3–7]

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In addition, it is remarkably safe compared to the most toxic antiprotozoal products.[8]

MTZ and the related nitroimidazole tinidazole (which is not available in some countries) are the only drugs effective for the treatment of trichomoniasis. In the event of overt clinical resistance to metronidazole in the anaerobic protozoa, there is no alternative treatment for either trichomoniasis or invasive amoebiasis, keeping in mind the documented cross-resistance between currently used nitroimidazole drugs and worldwide availability.[7] In this sense, at present this chemical has shown important strain-resistance effects.[9–11] However, the great cost associated with the development of new compounds and the small economic size of the market for antiprotozoal drugs make this development slow.

In addition to in vitro and in vivo tests, which are very expensive and time-consuming, powerful methods for 'rational' drug design and lead-like compound dataset screening and selection are available now.[12–17] In this sense, our research group has recently developed simple non-stochastic and stochastic molecular descriptors based on algebra theory. They have been defined in analogy to the quadratic and linear mathematical maps.[16,17] Applications included the prediction of several physical, physicochemical, chemical, and pharmacokinetical properties of organic compounds.[18,19] In addition, these indices have been extended to consider three-dimensional features of small/medium-sized molecules based on the trigonometric 3D-chirality correction factor approach.[20] Promising results have also been obtained in the selection of novel subsystems of compounds having a desired activity. For instance, it was successfully applied to the virtual screening of novel anthelmintic compounds, which were then synthesized and evaluated in vivo on *Fasciola hepatica*.[21] Studies for the fast-track discovery of novel paramphistomicides and antimalarial compounds were also conducted with this theoretical approach.[22,23]

The main aim of this study was to develop new QSAR models, based on statistical linear discriminant analysis (LDA) and non-stochastic (and stochastic) atom-type quadratic indices to predict the antiprotozoal activity and therefore, find rationality in the search of novel trichomonacidal drugs. This approach permitted the classification of candidate compounds as active (antitrichonals)/inactive previous to the pharmacological screenings, identifying the best candidates to be evaluated from thousand of compounds. Finally, the chemicals found were submitted to standard antiprotozoan tests to corroborate their theoretical activity.

The first step in the search of good LDA-based QSAR models is to use a dataset with a great molecular diversity. To ensure this molecular diversity, we have selected a data set of 107 compounds, 47 of them used as trichomonacidal and the rest (60 chemicals) having a series of other pharmacological uses.[24] These compounds were split into training and test sets that include 90 and 17 molecules, respectively.

Later, the molecular structure of each compound in dataset was coded using local (atom-type) non-stochastic and stochastic quadratic indices.[16,18–23] The *k*th atom-type quadratic indices are calculated by adding the *k*th atomic quadratic indices for all atoms of the same type in the molecule. In the atom-type quadratic indices formalism, each atom in the molecule is classified into an atom-type (fragment), such as heteroatoms, halogen atoms, aliphatic carbon chain, aromatic atoms (aromatic rings), and so on. The mathematical basis and a methodological explanation about the use of this approach have recently been described in previous reports.[16,18–23] In this study, specifically we used the *k*th ($k = 15$) atom-type (heteroatoms: S, N, O) quadratic fingerprints not considering and considering H-atoms in the molecular pseudograph (G), correspondingly [$\boldsymbol{q}_{k\mathrm{L}}(x_{\mathrm{E}})$ and $\boldsymbol{q}_{k\mathrm{L}}{}^{\mathrm{H}}(x_{\mathrm{E}})$]. These molecular descriptors were calculated with TOMOCOMD-CARDD software.[15]

LDA is a useful technique to find discriminant functions with the ability to distinguish between two groups or populations.[25] To derive discriminant functions that permit the classification of lead-like compounds as positive (presence of trichomonacidal activity) or negative (absence of trichomonacidal activity), we used LDA in which non-stochastic and stochastic heteroatoms' quadratic indices were used as independent variables. For obtaining LDA-based QSAR models, we used the statistic package STATISTICA.[26] Forward stepwise procedure was fixed as the strategy for variable selection and the principle of parsimony (Occam's razor) was taken into account as strategy for model selection. The quality of the models was determined by examining Wilks' $\lambda$ parameter ($U$-statistic), square Mahalanobis distance ($D^2$), Fisher ratio ($F$) and the corresponding $p$-level ($p(F)$) as well as the percentage of good classification in the training and test sets. The classification obtained models are given below together with the LDA-statistical parameters:

**Table 1.** Prediction performances for two LDA-based QSAR models

| | Matthews Corr. Coefficient ($C$) | Accuracy '$Q_{\mathrm{Total}}$' (%) | Sensitivity 'hit rate' (%) | Specificity (%) | False positive rate 'false alarm rate' (%) |
|---|---|---|---|---|---|
| Non-stochastic descriptors Eq. 1 | | | | | |
| Training set | 0.89 | 94.44 | 87.18 | 100.00 | 8.93 |
| Test set | 0.75 | 88.24 | 87.50 | 87.50 | 11.11 |
| Stochastic descriptors Eq. 2 | | | | | |
| Training set | 0.73 | 86.67 | 84.62 | 84.62 | 11.76 |
| Test set | 0.75 | 88.24 | 87.50 | 87.50 | 11.11 |

**Table 2.** Classification of compounds in the training set by use of the discriminant functions obtained by LDA

| Name | $\Delta P\%$[a] | $\Delta P\%$[b] |
|---|---|---|
| *Training active group* | | |
| Anisomycin | −94.39 | −46.96 |
| Virustomycin A | 93.85 | 17.89 |
| Azanidazole | 100.00 | 98.92 |
| Carnidazole | 98.36 | 95.43 |
| Propenidazole | 98.51 | 99.35 |
| Lauroguadine | −97.87 | −78.64 |
| Mepartricin A | 85.44 | 89.65 |
| Metronidazole | 97.33 | 97.22 |
| Nifuratel | 99.99 | 99.95 |
| Nifuroxime | 99.86 | 99.96 |
| Nimorazole | 99.14 | 99.55 |
| Secnidazole | 97.99 | 96.98 |
| Cariolin | 59.04 | −85.55 |
| 2-Amino-5-nitrotiazola | 92.97 | 97.35 |
| Glycobiarzol | 95.24 | 93.78 |
| Clioquinol | −48.10 | −65.67 |
| Diyodohydroxy-quinolina | −51.66 | −67.19 |
| Ornidazol | 99.86 | 97.45 |
| Trichomonacid | 99.49 | 99.96 |
| Lutenurine | 30.12 | −25.97 |
| Abunidazole | 99.76 | 97.60 |
| Imoctetrazoline | 99.74 | 78.96 |
| Forminitrazole | 92.63 | 97.64 |
| Chlomizol | 96.98 | 91.19 |
| Acinitrazole | 92.39 | 98.69 |
| Moxnidazole hydrocloride | 100.00 | 99.94 |
| Isometronidazole | 96.21 | 97.90 |
| Mertronidazole phosphate | 99.44 | 99.96 |
| Benzoylmetroni-dazole | 99.63 | 99.33 |
| Bamnidazole | 94.95 | 96.50 |
| Glycarsiamidon | 7.70 | 30.38 |
| Fexinidazole | 99.93 | 99.27 |
| Piperanitrozole | 99.01 | 98.82 |
| Gynotabs | 99.70 | 99.43 |
| Pirinidazole | 99.97 | 99.07 |
| Metronidazole hydrogen succinate | 98.49 | 99.06 |
| Tolamizol | 98.22 | 99.38 |
| Thiacetarsamide | −54.04 | 6.20 |
| Tivanidazole | 99.99 | 97.13 |
| *Training inactive group* | | |
| Amantadine | −99.46 | −94.28 |
| Thiacetazone | −36.93 | −26.86 |
| Cloral betaine | −92.91 | −97.44 |
| Carbavin | −99.64 | −87.04 |
| Norantoin | −90.60 | −93.87 |
| Orotonsan Fe | −97.84 | −79.17 |
| Picosulfate | −97.37 | 98.90 |
| Naftazone | −36.37 | −60.47 |
| Besunide | −99.70 | 71.05 |
| Acetazolamide | −86.07 | 22.68 |
| Propamin soviet | −99.75 | −95.59 |
| RMI 11894 | −96.77 | −96.29 |
| Ag 307 | −97.11 | −84.64 |
| Barbismetylii iodidum | −96.02 | −78.43 |
| Pancuronium bromide | −86.30 | 22.86 |
| Vinyl ether | −99.14 | −90.83 |
| Basedol | −95.23 | −89.91 |
| Carbimazole | −90.24 | −90.08 |
| Didym levulinate | −99.35 | −85.61 |
| Percloroetane | −1.64 | −96.32 |
| Pyrantel tartrate | −70.33 | −94.79 |
| Fentanilo | −93.02 | −68.33 |
| Petidina | −98.32 | −71.15 |
| Tenalidine tartrate | −95.56 | −76.40 |
| Bamipine | −94.45 | −80.78 |
| Colestipol | −99.94 | −96.22 |
| Non-aferone | −83.80 | −73.33 |
| Rolipram | −88.94 | −44.61 |
| *N*-Hydroxymethyl-*N*-methylurea | −99.85 | −98.83 |
| 4-Clorobenzoic acid | −95.02 | −65.12 |
| Acetanilide | −97.65 | −85.89 |
| Guanazole | −99.93 | −94.07 |
| Tetramin | −98.98 | −87.00 |
| Mecysteine | −98.85 | −98.90 |
| Cirazoline | −76.07 | −91.14 |
| Methocarbamol | −95.58 | −84.82 |
| Lysergide | −74.07 | −70.16 |
| Dopamine | −95.86 | −91.45 |
| Bufeniode | −83.88 | −74.07 |
| Celiprolol | −91.63 | −22.27 |
| Erysimin | −72.66 | 24.64 |
| Peruvoside | −28.13 | 42.60 |
| Amitraz | −17.99 | −97.28 |
| Proclonol | −75.60 | −77.75 |
| Asame | −56.42 | −76.53 |
| KC-8973 | −89.34 | −80.56 |
| Ethydine | −96.89 | −70.92 |
| Magnesii metioglicas | −95.78 | −89.74 |
| Alibendol | −96.80 | −69.43 |
| Diponium bromide | −98.12 | −56.84 |
| Streptomycin | −30.19 | −72.15 |

[a,b]Antitrichomonal activity predicted by Eqs. 1 and 2 using non-stochastic and stochastic atom-type quadratic indices, respectively. $\Delta P\% = [P(\text{Active}) - P(\text{Inactive})] \times 100$.

$$\begin{aligned} \textbf{\textit{Class}} &= -5.6 + 0.034\boldsymbol{q}_{3\text{L}}(x_{\text{E}}) - 0.0066\boldsymbol{q}_{4\text{L}}{}^{\text{H}}(x_{\text{E}}) \\ &\quad - 3.63 \times 10^{-8}\boldsymbol{q}_{14\text{L}}(x_{\text{E}}) + 2.54\mathbf{q}_{14\text{L}}{}^{\text{H}}(x_{\text{E}}) \\ N &= 90\ \lambda = 0.33\ D^2 = 8.07\ F(4,85) = 43.752 \\ p &< 0.0001 \end{aligned} \tag{1}$$

$$\begin{aligned} \textbf{\textit{Class}} &= -3.83 + 0.47^{s}\boldsymbol{q}_{1\text{L}}(x_{\text{E}}) \\ &\quad - 0.76^{s}\boldsymbol{q}_{6\text{L}}{}^{\text{H}}(x_{\text{E}}) + 0.45^{s}\boldsymbol{q}_{4\text{L}}(x_{\text{E}}) \\ N &= 90\ \lambda = 0.40\ D^2 = 5.94\ F(34,86) = 44,999 \\ p &< 0.0001 \end{aligned} \tag{2}$$

The classification of cases was performed by means of the posterior classification probabilities. By using the models, one compound can then be classified as active if $\Delta P\% > 0$, being $\Delta P\% = [P(\text{Active}) - P(\text{Inactive})] \times 100$, or as inactive otherwise. $P(\text{Active})$ and $P(\text{Inactive})$ are the probabilities with which the equations classify a compound as active and inactive, respectively.

The results of global good classification of compounds [accuracy ($Q_{\text{total}}$)], in both training and test sets, are shown in Table 1. As it can also be computed from the results shown in Tables 2 and 3, models **1** and **2** correctly classified 94.44% (88.24%) and 86.67% (88.24%) of the whole training (test) sets, respectively (see accuracy in Table 1). Table 1 also lists most of the parameters

**Table 3.** Classification of compounds in the test set by use of the discriminant functions obtained by LDA

| Name | $\Delta P\%^a$ | $\Delta P\%^b$ |
|---|---|---|
| *Test active group* | | |
| Furazolidone | 99.98 | 99.80 |
| Mepartircin B | 88.10 | 84.70 |
| Aminitrozole | 92.39 | 98.69 |
| Clotrimazol | −4.77 | −78.45 |
| Azomycin | 100.00 | 99.11 |
| Ternidazole | 96.60 | 98.41 |
| Misonidazole | 99.64 | 99.31 |
| Satranidazole | 99.47 | 95.67 |
| *Test inactive group* | | |
| Methenamine | −98.35 | −89.82 |
| Phenoltetrachlorophthalein | −92.19 | 57.12 |
| Carazolol | −91.95 | −79.82 |
| Frigen 113 | 40.47 | −99.11 |
| Eticoumarolum | −92.81 | −12.52 |
| Ciclopramine | −91.72 | −90.21 |
| Trimetilsulfonium hydroxide | −99.65 | −81.31 |
| Zoxazolamine | −92.49 | −68.97 |
| Acetylcholine | −99.64 | −54.67 |

[a,b]Antitrichomonal activity predicted by Eqs. 1 and 2 using non-stochastic and stochastic atom-type quadratic indices, respectively. $\Delta P\% = [P(\text{Active}) - P(\text{Inactive})] \times 100$.

commonly used in 'medical' statistics [sensitivity, specificity (also known as 'hit rate'), false positive rate (also known as 'false alarm rate') and the Matthews correlation coefficient (***C***)] for both obtained models.[35] These models, Eqs. (1) and (2), showed a high ***C*** of 0.89 (0.75) and 0.73 (0.75) in training (test) sets, correspondingly. While the sensitivity is the probability of correctly predicting a positive example, the specificity is the probability that a positive prediction is correct. On the other hand, ***C*** quantifies the strength of the linear relation between the molecular descriptors and the classifications, and it may often provide a much more balanced evaluation of the prediction than, for instance, the percentages.[27]

Since tests above simulated the situation of virtual screening, the particular ability to select compounds from a never used dataset demonstrates the effectiveness of this approach for the computational high throughput in silico screening of trichomonacidal agents. No previous reports related to the application of pattern recognition techniques to the selection of trichomonacidal compounds from a heterogeneous series of compounds were found in the literature. Therefore, the present algorithm constitutes a step forward in the search of efficient ways to discover new drugs bioactive against *T. vaginalis*. In this sense, we can outline that in silico 'virtual' screening is a complementary alternative to the 'real' world of synthesis and screening of chemicals in the laboratory. This *biosilico* world of data, analysis, hypothesis, identification and design/optimization resides inside of a computer and by this means the synthesis and bioassay are made only after exploring the initial concepts with QSAR models.[28]

To test the potential of TOMOCOMD-CARDD method and LDA for detecting novel antiprotozoal leads, we predicted the biological activity of all the chemicals contained in our 'in-house' collections of indazole, indole, cinnoline and quinoxaline derivatives which have recently been obtained by some of our research groups.[2,29–36] After applying the LDA-based QSAR models to different structures contained in these heterocyclic collections (100 compounds), we have selected a group of eight among those with higher probability of antitrichomonal action (see Fig. 1) as theoretically active chemicals to be tested in an in vitro assay.

The method used for the assessment of antiprotozoa activity of the selected molecules was an in vitro trichomonacidals test.[37–40] The results of the activity against *T. vaginalis* of the compound study objects are shown in Table 4. These outcomes exemplify how the present approach could be used for the selection/identification of new antitrichomonal drug candidates. Our trained LDA-based QSAR models successfully classified nine out of ten compounds to be active yielding an accuracy of the 87.50% (7/8).

In general, the compounds VA7-67 and VA7-69 that belong to the series 1,4 susbstituted 7- nitroquinoxalin-2 ones maintain their efficacy at 10 μg/ml with an important trichomonacidal activity [100.00% (100.00%) and 92.03% (93.94) of reduction activity, respectively]. It is remarkable that the compound VA7-67 did not show toxic activity in macrophage cultivations but their coun-

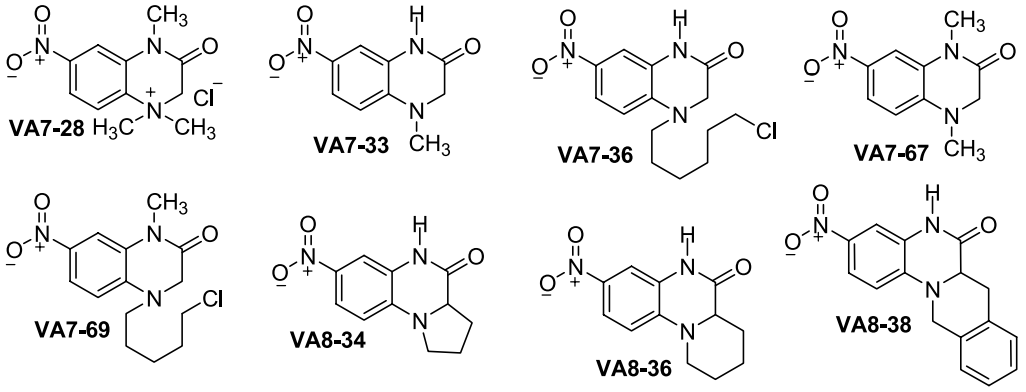


**Figure 1.** Chemical structures of the assayed chemicals.

**Table 4.** Results of the computational evaluation using LDA-based QSAR models and percentages of cytostatic and/or cytocidal activity [brackets] for the three concentrations assayed in vitro against *Trichomonas vaginalis*

| Compound* | Theoretical results | | | | In vitro activity (μg/ml)[f] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Class[a] | $\Delta P\%$[b] | Class[c] | $\Delta P\%$[d] | Class[e] | $\%CA_{24\ h}$ [$\%C_{24\ h}$] | | | $\%CA_{48\ h}$ [$\%C_{48\ h}$] | | |
| | | | | | | *100* | *10* | *1* | *100* | *10* | *1* |
| VA7-28 | + | 99.38 | + | 99.82 | + | **[100.0]** | 72.85 | 0.17 | **[100.0]** | 1.13 | 0.78 |
| VA7-33 | + | 98.88 | + | 99.78 | + | **[100.0]** | 59.05 | 0.00 | **[100.0]** | 13.97 | 8.32 |
| VA7-36 | + | 98.92 | + | 99.91 | + | **[99.15]** | 79.28 | 8.76 | **[99.83]** | 30.56 | 0.00 |
| VA7-67 | + | 99.04 | + | 99.78 | + | **[100.0]** | 71.11 | 0.00 | **[100.0]** | **[92.03]** | 0.00 |
| VA7-69 | + | 99.06 | + | 99.90 | + | **[100.0]** | 72.68 | 0.00 | **[100.0]** | **[93.94]** | 69.96 |
| VA8-34 | + | 99.59 | + | 99.42 | + | **[59.99]** | 33.48 | 11.09 | **[100.0]** | 54.22 | 17.32 |
| VA8-36 | + | 99.58 | + | 99.43 | + | **[98.43]** | 57.45 | 0.00 | **[100.0]** | 75.15 | 0.00 |
| VA8-38 | + | 99.79 | + | 99.77 | – | 85.53 | 51.82 | 0.00 | 81.25 | 37.15 | 0.00 |
| MTZ | + | 98.49 | + | 99.06 | + | **[99.63]** | **[99.18]** | **[98.19]** | **[100.0]** | **[99.72]** | **[98.79]** |
| Accuracy | | 87.50% | | 87.50% | | | | | | | |

[a,c]In silico classification obtained from model **1** and **2** using non-stochastic and stochastic atom-type quadratic indices, respectively.
[b,d]Results of the classification of compounds obtained from model **1** and **2**, correspondingly: $\Delta P\% = [P(\text{Active}) - P(\text{Inactive})] \times 100$.
[e]Observed (experimental activity) classification against *T. vaginalis*.
[f]Pharmacological activity of each of the tested compounds which were added to the cultures at doses of 100, 10, and 1 μg/ml: $\%CA_{\#}$ = cytostatic activity$_{(24–48\ h)}$ and [$\%\boldsymbol{C}_{\#}$] = cytocidal activity$_{(24–48\ h)}$. MTZ, metronidazole (concentrations for metronidazole were 2, 1, and 0.5 mg/ml, respectively).
* The molecular structure of the compounds represented with codes are shown in Figure 1.

terpart (compound VA7-69), with similar activity against *T. vaginalis*, showed bigger cytotoxic activity than the previous ones. Nevertheless, this side effect is considered inside the standard parameters.[40]

Most of the other compounds showed high activity against *T. vaginalis* at the concentration of 100 μg/ml and low cytotoxicity at this concentration. Even so, none of the compounds studied were more active than metronidazole. In this sense, we are looking forward to improving these results in the future by finding more potent candidates. However, our current results are significant because they demonstrate the straightforward way in which TOMOCOMD-CARDD method can identify new trichomonacidal lead organic-chemical drugs. These results open at the same time a door for the study of this family of heterocyclic compounds, which seems to be a promising source of antiprotozoal drugs. Current investigations in this direction are now in progress and they will be subject to future publications in forthcoming papers.

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40. The selected compounds were assayed in vitro on *T. vaginalis* strain JH31A (ATCC, Maryland, USA) in modified Diamond medium supplemented with equine serum and grown at 37 °C (5% $CO_2$). The compounds were added to the cultures at several concentrations (100, 10, and 1 μg/ml) after 6 h of the seeding (0 h). Viable protozoa were assessed at 24 and 48 h after incubation at 37 °C by using the Neubauer chamber. Metronidazole (Sigma–Aldrich SA, Spain) was used as reference drug at concentrations of 2, 1, and 0.5 μg/ml. By means of microscopic counts, cytostatic (percentage of growth inhibition) and cytocidal (percentage of reduction) activities were determined with respect to controls as previously reported.