



## METHOD ARTICLE

# Virtual-screening workflow tutorials and prospective results from the Teach-Discover-Treat competition 2014 against malaria [version 1; referees: 2 approved]

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**v1** First published: 17 Jul 2017, 6:1136 (doi: [10.12688/f1000research.11905.1](https://doi.org/10.12688/f1000research.11905.1))  
Latest published: 17 Jul 2017, 6:1136 (doi: [10.12688/f1000research.11905.1](https://doi.org/10.12688/f1000research.11905.1))

## Abstract

The first challenge in the 2014 competition launched by the Teach-Discover-Treat (TDT) initiative asked for the development of a tutorial for ligand-based virtual screening, based on data from a primary phenotypic high-throughput screen (HTS) against malaria. The resulting Workflows were applied to select compounds from a commercial database, and a subset of those were purchased and tested experimentally for anti-malaria activity. Here, we present the two most successful Workflows, both using machine-learning approaches, and report the results for the 114 compounds tested in the follow-up screen. Excluding the two known anti-malarials quinidine and amodiaquine and 31 compounds already present in the primary HTS, a high hit rate of 57% was found.

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<b>version 1</b> published 17 Jul 2017	 report	 report

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**Competing interests:** The authors declare no competing financial interests.

**How to cite this article:** Riniker S, Landrum GA, Montanari F *et al.* **Virtual-screening workflow tutorials and prospective results from the Teach-Discover-Treat competition 2014 against malaria [version 1; referees: 2 approved]** *F1000Research* 2017, 6:1136 (doi: [10.12688/f1000research.11905.1](https://doi.org/10.12688/f1000research.11905.1))

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**Grant information:** SR thanks the Novartis Institutes for BioMedical Research education office for a Presidential Postdoctoral Fellowship. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

**First published:** 17 Jul 2017, 6:1136 (doi: [10.12688/f1000research.11905.1](https://doi.org/10.12688/f1000research.11905.1))

## Introduction

Teach-Discover-Treat (TDT) is an initiative that aims to provide high-quality tutorials of important tasks in computer-aided drug discovery, in order to impact education and drug discovery for neglected diseases<sup>1</sup>. The TDT steering committee consists of computational chemists from both academia and industry. To encourage the creation of high-quality tutorials by the computational chemistry community, competitions are launched with a series of different challenges, and the results/tutorials are made available through the website of the initiative (<http://www.tdtproject.org>). The competitions are open to everybody. After the first successful competition in 2012<sup>2</sup>, a second competition was launched in 2014, with four challenges. In this study, we focus on Challenge 1: ligand-based virtual screening (VS) against malaria. The goal was to build a predictive model for anti-malaria activity based on a phenotypic high-throughput screen (HTS), and to use that model subsequently to select the next set of compounds for screening. In a ligand-based VS, typically no structural information of the target is available, and thus the prediction of potentially active compounds is based on the principle that similar molecules exhibit similar activity<sup>3</sup>. The challenge is thereby to find an appropriate molecular description for similarity, which can depend heavily on the compound selection and/or target<sup>4-7</sup>. In recent years, machine-learning (ML) methods have emerged as an attractive tool to boost the predictive power of ligand-based VS approaches<sup>8-12</sup>.

Malaria is caused in humans by several species of the protozoan parasite *Plasmodium*. The most lethal species is *Plasmodium falciparum* (Pf), which causes organ failure and accumulates in the brain capillaries if left untreated. Malaria is still one of the most prevalent and deadly diseases in Africa, Asia and the Americas, with an estimate of 198 million cases in 2013 leading to approximately 584,000 deaths according to the 2014 world malaria report of the World Health Organization (WHO)<sup>13</sup>. Recent advances in malaria research and drug discovery have been reviewed<sup>14-19</sup>. The anti-malaria drugs can be broadly classified into three groups: (i) compounds that interfere with the heme detoxification, (ii) compounds that target folate metabolism, and (iii) compounds that inhibit mitochondrial electron transport. The current standard of care for uncomplicated malaria is artemisinin-based combination therapies. Artemisinins belong to the third group of anti-malaria drugs and rapidly kill all the blood stages of the parasite, however, they are also cleared in a short time<sup>20</sup>. Unfortunately, the emergence of resistant strains has become a major problem in recent years<sup>21,22</sup>, requiring the development of new and possibly orthogonal drugs. In the past, whole-cell phenotypic screening campaigns against Pf have been successful in identifying new lead compounds<sup>23</sup>.

Challenge 1 of the 2014 TDT competition involved three tasks: (i) analysis of the data from a single-concentration phenotypic HTS of 305,568 compounds, including hit-list triaging and selection

of compounds for a follow-up screen with EC<sub>50</sub> measurement, (ii) building and validation of a predictive anti-malaria activity model, including a held-out test-set of 1056 compounds, and (iii) follow-up hit finding by applying the predictive model to rank-order a large dataset of commercially available compounds. The top 1000 molecules of this ranked list were considered further for experimental testing. For training, the challenge provided results for 305,568 compounds from the primary HTS, as well as EC<sub>50</sub> data from a follow-up confirmatory screen for a subset of the compounds.

In this study, we present the results of two Workflows. Workflow 1 was the overall winner of the competition, and Workflow 2 showed the best performance on the held-out test set measured in the phenotypic Pf screen. Note that the two Workflows interpreted the challenge differently. In Workflow 1, only data from the primary HTS was used in the training of the predictive model in order to mimic the early phase of a drug discovery campaign. In Workflow 2, the EC<sub>50</sub> data from the confirmatory assay was taken into account in order to improve the labelling of the training set. Each Workflow provided a ranked list of the top 1000 molecules, from which a total of 114 compounds (80 from Workflow 1 and 38 from Workflow 2, four were in common) were selected based on vendor availability for screening in a Pf phenotypic assay. Excluding the two known anti-malarials quinidine and amodiaquine and the 31 compounds already present in the primary HTS, 46 of 81 compounds were found to be active in the follow-up assay, which corresponds to a hit rate of 57%.

## Methods

The basis for the virtual screening workflows was a phenotypic high-throughput screen against Pf with 305,568 compounds, together with a confirmatory dose-response screen for 1524 compounds, which are reported in 23. The data is deposited in ChEMBL as part of the Neglected Tropical Diseases set (ChEMBL-NTD)<sup>24</sup>. The data is also available on the TDT website (<http://www.tdtproject.org/challenge-1---malaria-hts.html>). In addition, an external held-out test set with 1056 molecules was provided for comparison of submissions<sup>25</sup>. This dataset was generated in the laboratory of R. K. Guy in 2014, following the same procedure as described in 23, at the time of the TDT competition. Results for this held-out set are given in the [Supplementary material](#).

## Workflow 1

The tutorial was written in the form of an IPython notebook and a series of Python scripts for the computationally demanding tasks to be executed separately. The tutorial is available on the TDT website (<http://www.tdtproject.org>) and on GitHub (<https://github.com/sriniker/TDT-tutorial-2014>). The tutorial makes use of a number of open-source Python libraries: the cheminformatics toolkit RDKit version 2013.09 (<http://www.rdkit.org>), the machine-learning toolkit scikit-learn version 0.13 (<http://scikit-learn.org>), pandas for working with data tables, and libraries for scientific computing,

numpy version 1.6.2 and scipy version 0.9.0. Figures are plotted using matplotlib version 1.1.0. The components of the Workflow are shown schematically in [Figure 1](#).

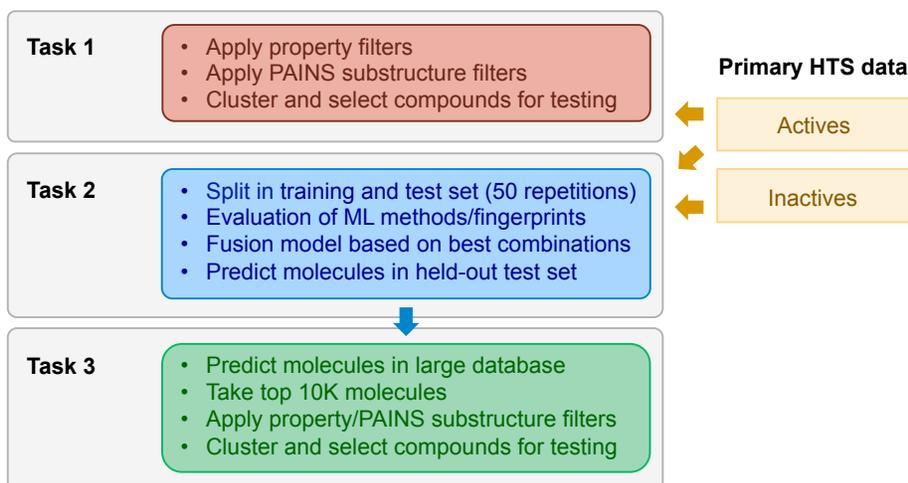
### Data preprocessing

The input for the workflow was the hit list from the phenotypic HT screen, with a classification into ‘active’, ‘inactive’, and ‘ambiguous’ compounds<sup>23</sup>. From the original 305,568 compounds tested in the screen, 1528 were found to be active and 293,608 inactive. The 10,432 molecules with an ambiguous outcome were discarded.

### Task 1: Selection of 500 molecules for follow-up testing

To triage the hit list in the first task, property filters ([Table 1](#)) based on previously described filters<sup>26,27</sup> were applied for *in silico* post-processing of the primary HTS data, which resulted in 1512 remaining active compounds.

Next, the active molecules were checked for potentially problematic substructures using the PAINS filters described in [28](#). 1225 molecules passed these filters. From these, 500 molecules had to be picked for testing in a confirmatory assay. While making this selection, a balance between the desire to have a good sampling of the chemical space covered by the primary actives and the desire to get some structure-activity relationship (SAR) information from the confirmatory assay had to be found. The compounds were therefore clustered using the Butina algorithm<sup>29</sup> based on Tanimoto similarity with a cutoff = 0.5. The Tanimoto similarity was calculated using RDKit fingerprints (a subgraph-based fingerprint similar to the Daylight fingerprint), with a maximum path length of five. 304 clusters were found, with only 40 clusters having more than five members. The cluster centers provide a set of diverse seeds. To ensure the chance to obtain information about SAR, molecules around the cluster centers were selected:



**Figure 1.** Schematic representation of Workflow 1.

**Table 1.** Property filters for *in silico* post-processing of primary HTS data. These filters are used in Workflow 1.

Property	Range
Molecular weight	100–700 g/mol
Number of heavy atoms	5–50
Number of rotatable bonds	0–12
Hydrogen-bond donors	0–5
Hydrogen-bond acceptors	0–10
Hydrophobicity	-5 < logP < 7.5

Starting with the largest cluster, the five molecules most similar to the cluster center (or 50% of the cluster members if the cluster contained less than 5 molecules) were picked.

### Task 2: Prediction of anti-malarial activity for the held-out test set

Three different machine-learning (ML) models together with three different molecular fingerprints were tested for the predictive model in task 2. The ML methods were random forest (RF)<sup>30</sup>, Naive Bayes (NB) and logistic regression (LR), which showed a good performance in a previous benchmarking study<sup>13</sup>. The RF models were built using 100 trees, a maximum depth of 100, and minimum one sample in a leaf. For NB and LR, the default parameters in scikit-learn were used. The fingerprints were atom pairs (AP)<sup>31</sup>, RDKit fingerprint with a maximum path length of five (RDKit5) and Morgan fingerprint with a radius of 2 (Morgan2)<sup>32</sup>, and are described in more detail in 8. In the version of the Workflow submitted in the competition, the AP and RDKit5 fingerprints were hashed to 2048 bits, and the Morgan2 fingerprints to 1024 bits. Later on we found that a fingerprint size of 4096 bits resulted in better performances due to fewer collisions. To determine which ML method/fingerprint combinations performed best and should therefore be combined using heterogeneous classifier fusion<sup>13</sup>, a retrospective evaluation was performed using the primary HTS data. Here, all data points from the primary screen were used (i.e. none of the property-/substructure-filters discussed above were applied) as some filters may be too strict and the ML methods are rather robust to noise. The data points were randomly split 50 times into a training set (90%) and a test set (10%). A ML model was built using the training set and the molecules in the test set were ranked based on the predicted probability to be active. From the ranked list, the receiver operating characteristic (ROC) curve was calculated and subsequently the area under the ROC curve (AUC) was determined. In addition, the enrichment factor at 5% was determined. A detailed discussion of the different evaluation methods is given in 8. The results from the retrospective evaluation, averaged over the 50 repetitions, are listed in Table 2. Based on these results and the analysis of the diversity in the active molecules that were identified, a classifier fusion model was proposed based on RF with RDKit5, RF with Morgan2 and LR with RDKit5 (Table 2). As a last step, a fusion model was trained using all data points of the primary HTS in order to obtain predictions for the held-out test set and for task 3.

### Task 3: Selection of 1000 new candidates from the eMolecules catalog

In task 3, the goal was to select a list of 1000 compounds from the eMolecules (<https://www.emolecules.com>) catalog, with nearly 5.5 million commercially available compounds. As a first step, the molecules were filtered using the property filters described in Table 1 except logP. logP was not applied at this stage to reduce the computational cost. This resulted in approximately 4.4 million compounds. For these, molecular fingerprints (RDKit5 and Morgan2) were generated with 4096 bits and the anti-malaria activity was

predicted using the fusion model trained on the primary HTS in task 2. The top ranked 10,000 compounds were taken for further selection. The logP filter (see Table 1) and PAINS substructure filters were applied at this point. Filtering resulted in 7955 compounds. To select 1000 molecules from these, the following procedure was applied:

- The highest-ranked molecule is selected as first cluster center.
- Taking the next lower molecule, the similarity to the first molecule is calculated:
  - If the similarity is below 0.5, the molecule is selected as a new cluster center.
  - If the similarity is above 0.85 and the cluster does not contain 6 molecules yet (including the cluster center), the molecule is selected and added to the cluster.
  - Else the molecule is discarded.

The procedure was continued until 1000 compounds were selected. Unfortunately, a bug in the selection step of the original tutorial resulted in the 1000 compounds being randomly selected from the top ranked 10,000 compounds. In addition, compounds already in the primary HTS used for training were not explicitly removed from the eMolecules catalog. A corrected version of

**Table 2. Evaluation results for anti-malaria activity prediction using a 90%-training and 10%-test set split for Workflow 1.**

The random selection was repeated 50 times and the results were averaged over the repetitions. The maximum possible EF5% value is 20.0. Fingerprints with 4096 bits were used.

Method	AUC	STD AUC	EF5%	STD EF5%
Similarity AP	0.88	0.02	13.94	0.69
Similarity RDKit5	0.88	0.02	13.75	0.74
Similarity Morgan2	0.89	0.02	14.65	0.69
NB with AP	0.80	0.02	7.40	0.64
NB with RDKit5	0.81	0.02	8.27	0.80
NB with Morgan2	0.85	0.02	10.42	0.98
LR with AP	0.88	0.02	12.53	0.92
LR with RDKit5	0.91	0.02	14.99	0.80
LR with Morgan2	0.88	0.02	13.30	0.75
RF with AP	0.92	0.01	14.66	0.75
RF with RDKit5	0.93	0.02	15.38	0.70
RF with Morgan2	0.93	0.01	15.28	0.70
Fusion model	0.93	0.01	15.75	0.73

the tutorial is provided on GitHub (<https://github.com/sriniker/TDT-tutorial-2014>).

## Workflow 2

The tutorial is available on the TDT website (<http://www.tdtproject.org>) and on GitHub (<https://github.com/sdvillal/tdt-malaria-followup>). RDKit version 2013\_09\_2 (<http://www.rdkit.org>) was used to read the SMILES strings, compute descriptors and fingerprints. Scikit-learn version 0.14 (<http://scikit-learn.org>) was used to build the models.

## Data preprocessing

The input was again the original primary HTS data<sup>23</sup> with 1528 active compounds, 293,608 inactive compounds and 10,432 molecules with an ambiguous outcome. In addition, pEC<sub>50</sub> data from a dose-response confirmatory screen for 1524 compounds<sup>23</sup> was taken into account. Compounds were relabeled using, when available, the confirmatory pEC<sub>50</sub> data. Any compound with a pEC<sub>50</sub> of at least 5 was considered positive for anti-malarial activity independent of the original classification. As a result, 296 molecules were relabeled from positive to negative; 192 molecules were relabeled from ambiguous to negative; 52 molecules were relabeled from ambiguous to positive; 4 molecules were relabeled from negative to positive. The final dataset contained 1288 compounds labeled as positives, 294,092 as negatives, and 10,188 as ambiguous. Ambiguous compounds were not considered for modeling.

## Descriptors and unfolded circular fingerprints

To describe the chemical structures of the compounds, the 196 RDKit descriptors available by default were computed. This first set will be referred to as “RDKit descriptors” set. Morgan fingerprints of both extended connectivity (ECFP) and functional class (FCFP) types<sup>32</sup> were computed with a radius of up to 200 (meaning that all possible substructures are enumerated for each compound). Typically, circular fingerprints are hashed and folded to a fixed size, but this may lead to collisions, i.e. two different substructures are hashed to the same bit in the folded fingerprint. To avoid this problem, hashing or folding was not used in Workflow 2. All the existing substructures were saved as SMILES strings and uniquely encoded by a large bitset containing all substructures occurring in the training set. The unfolded ECFP and FCFP fingerprints were appended together in one vector.

## Model building, validation and selection

Random forests<sup>29</sup> and extremely randomized trees<sup>33</sup> of 10, 20, 50, 100, 500, 1000, 2000, 4000 and 6000 trees were computed on the RDKit descriptors set, using multiple random seeds. Both methods use bagging to select instances for building each tree. As a result, for each individual tree, some instances were not used for training and are referred to as “out-of-bag”. These instances can be used for an unbiased estimate of the prediction error, instead of performing a computationally expensive cross-validation. Therefore, the out-of-bag scores were used as a measure of the quality of the models, and AUC, accuracy and enrichment at 5% were computed from these scores. The ensemble of trees with

6000 trees gave the best results and was therefore selected for deployment (i.e. used for the computation of the final scores for the unlabeled datasets).

After a first exploration of multiple parameters for logistic regression on the fingerprint set by cross-validation, the following parameters for building the models were chosen: a penalty of 11 or 12, a regularization parameter C of 1 or 5, a default tolerance of 0.0001, and the fingerprints were kept unfolded. Cross-validation was computed for 3, 5, 7 or 10 folds with five different seeds each. For each fold, the AUC and enrichment at 5% were computed. When a fold reached an AUC below 0.88, then the rest of the cross-validation was skipped and the next model was built.

The best models among the many logistic regressions models for which all folds could be completed were the ones with a penalty of 11 and C of 1 and an average AUC over all folds over 0.92; as well as those with a penalty of 12 and C of 5 and an average AUC over all folds over 0.93. These particular models were selected for deployment (i.e. used for the computation of the final scores for the three tasks).

## Task 1: Selection of 500 molecules for follow-up testing

The first task involved the selection of 500 molecules from the primary HTS set with promising activity for follow-up confirmatory measurements. For this, the predictions of the deployment models were combined by plain averaging of the model scores. Note that this corresponds to model fitting scores, since the screening set is the training set used for building the deployment models. The 500 molecules with the highest average scores were selected for the follow-up testing.

## Task 2: Prediction of anti-malarial activity for the held-out test set

In 1992, Wolpert introduced the concept of stacked generalization<sup>34</sup> to combine different models and boost the predictive power of the resulting ensemble. Here, feature-weighted linear stacking was used to combine our deployment models<sup>35</sup>. For this, a linear regression was trained using the out-of-bag scores (for the ensemble of trees models) and cross-validation scores (for the logistic regression models) as independent variable, and antimalarial activity as dependent variable. The resulting linear combination of models was applied to obtain the final score for the 1056 compounds of the held-out test set.

## Task 3: Selection of 1000 new candidates from the eMolecules catalog

For the selection of new candidates, the same feature-weighted linear stacking as described for Task 2 was used. The resulting linear combination of individual model scores was applied to obtain the final score for the compounds of the eMolecules catalog (<https://www.emolecules.com>). The 1000 top-scoring compounds were selected as new candidates for further anti-malaria screening. Compounds already present in the primary HTS and the confirmatory screen used for training were not explicitly removed from the eMolecules catalog.

## Final selection process

From the two lists of 1000 new candidates, 114 molecules were selected for testing in a follow-up assay based on availability at vendors who agreed to be TDT sponsors. The set included two known anti-malarials quinidine (proposed by Workflow 1) and amodiaquine (proposed by Workflow 2). Compounds that were already in the primary HTS and the confirmatory screen provided by the TDT challenge were not removed.

## Experimental procedures

The potency of new candidates was determined as reported earlier<sup>23</sup>. *Plasmodium falciparum* strain 3D7 was acquired from the Malaria Research and Reference Reagent Resource Center (MR4, catalog #MRA-102). Briefly, asynchronous parasites were maintained in culture based on the method of Trager<sup>36</sup>. Parasites were grown in presence of fresh group O-positive erythrocytes (Key Biologics, LLC, Memphis, TN) in Petri dishes at a hematocrite of 4-6% in RPMI based media (RPMI 1640 supplemented with 0.5% ALBUMAX II, 25 mM HEPES, 25 mM NaHCO<sub>3</sub> (pH 7.3), 100 µg/mL hypoxanthine, and 5 µg/mL gentamycin). Cultures were incubated at 37°C in a gas mixture of 90% N<sub>2</sub>, 5% O<sub>2</sub>, 5% CO<sub>2</sub>. For IC<sub>50</sub> determinations, 20 µl of RPMI 1640 with 5 µg/ml gentamycin were dispensed per well in an assay plate (Corning 384-well microtiter plate, clear bottom, tissue culture treated, catalog no. 8807BC). An amount of 60 nl of compound, previously serial diluted in a separate 384-well white polypropylene plate (Corning, catalog no. 8748BC), was dispensed to the assay plate by hydrodynamic pin transfer (FP1S50H, V&P Scientific Pin Head) and then an amount of 20 µl of a synchronized culture suspension (1% rings, 4% hematocrite) was added per well, thus making a final hematocrite and parasitemia of 2% and 1%, respectively. Assay plates were incubated for 72 h, and the parasitemia was determined by a method previously described<sup>37</sup>. An amount of 10 µl of the following solution in PBS (10X Sybr Green I, 0.5% v/v triton, 0.5 mg/ml saponin) was added per well. Assay plates were shaken for 1 min, incubated in the dark for 90 min, then read with the Envision spectrophotometer at Ex/Em of 485 nm/535 nm.

EC<sub>50</sub> values were calculated using a four-parameter logistic equation as described previously<sup>23</sup>. Compounds were arrayed in ten concentrations, varying from approximately 10 µM to 5 nM, and the R drc package was used to fit the observed response to the four-parameter Hill equation<sup>38</sup>. The purity of all compounds was determined by UPLC (UV and ELSD purity average) and results from any compound with a purity below 95% were not reported.

## Analysis

Morgan2 fingerprints<sup>32</sup> and Tanimoto similarities were calculated using the RDKit. The scaffolds in the set of newly tested compounds were determined using the Bemis-Murcko algorithm<sup>39</sup>.

## Results

### Held-out test set

The external held-out test set of the TDT challenge consisted of 101 actives and 955 inactives. The performances of the ML models of Workflow 1 and Workflow 2 on the held-out test set (1056 molecules) are given in Table 3. For Workflow 1, the results using fingerprints with 1024/2048 bits or with 4096 bits are reported. Note that the maximum possible EF5% for the held-out

test set is 10.5 (as the fraction  $\chi = 0.05$  is smaller than the ratio of actives to inactives<sup>8</sup>), whereas it is 20.0 for the primary HTS dataset. Workflow 2 gave the best performance for the held-out test set from all five submissions to this TDT challenge. For Workflow 1, the version using 1024/2048 bits was the one submitted to the TDT challenge. Later, it was found that a substantial amount of collisions due to hashing occurred in the short fingerprints, which affected the performance. Using longer fingerprints (i.e. 4096 bits), the performance could be improved and was found to be similar to that of Workflow 2. This highlights the resistance to noise of the ML methods used, since in Workflow 1 the false positives in the primary data were included. In Workflow 2, these false positives were corrected using the information from the confirmatory screen.

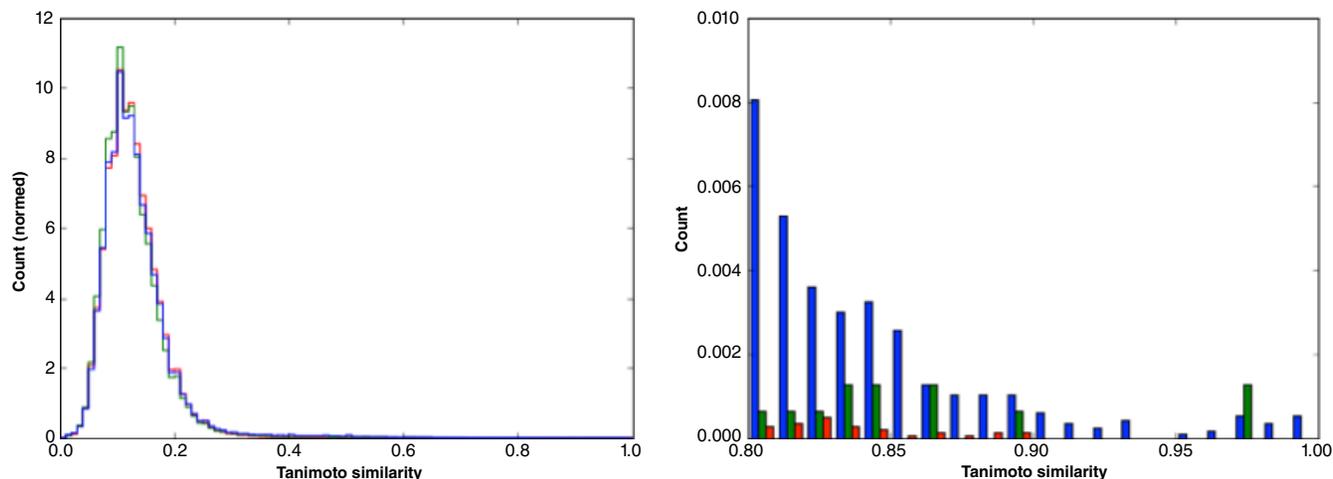
For both Workflows, the AUC and the EF5% values were found to be substantially lower for the held-out test set compared to the values for the 10%-test split in Table 2. Although the size distribution and flexibility of the compounds in the different sets were similar (Table 4) and the similarities within and across the datasets were generally low (left panel in Figure 2), there are slightly more highly similar compounds among the actives of the primary HTS (as in the original classification<sup>23</sup>) than between those and the actives in the held-out test set (right panel in Figure 2). In addition, there were

**Table 3. Evaluation results for anti-malaria activity on the held-out test set (1056 molecules).** Predictions were obtained using the fusion models of Workflow 1 and the linear combination of model scores of Workflow 2. The maximum possible EF5% value is 10.5.

Method	AUC	EF5%
Workflow 1 - Fusion model (1024/2048 bits)	0.74	2.76
Workflow 1 - Fusion model (4096 bits)	0.75	4.75
Workflow 2	0.79	4.34

**Table 4. Properties of the molecules in the primary HTS and in the held-out test set.** The compounds in the primary HTS were split into 1528 actives and 293,606 inactives. The compounds in the held-out test set were split into 101 actives and 955 inactives. For the primary screen, the original classification into actives and inactives was used<sup>23</sup>. For the held-out test set, a cutoff of 10 µM was employed.

Dataset	Median molecular weight [g/mol]	Median number of rotatable bonds
Actives in primary HTS	394.0	5.0
Inactives in primary HTS	373.1	5.0
Actives in held-out test set	387.2	5.0
Inactives in held-out test set	374.1	5.0



**Figure 2. Similarity distributions for the molecules in the primary HTS and the held-out test set.** Normalized Tanimoto similarity distribution using a Morgan2 fingerprint<sup>32</sup> within the actives in the primary HTS (blue), and between them and the actives (green) and inactives (red) of the held-out test set. The full distributions (left) and the slice between 0.8 and 1.0 similarity (right) are shown. For the primary screen, the original classification into actives and inactives was used<sup>23</sup>. For the held-out test set, a cutoff of 10  $\mu$ M was employed.

some highly similar compounds between the actives in the primary HTS and the inactives in the held-out test set.

### Prospective phenotypic screen

From the combined set of 2000 candidates predicted by Workflow 1 and Workflow 2, 114 were tested in a follow-up assay (80 from Workflow 1 and 38 from Workflow 2, four compounds were predicted by both Workflows). The identifiers, SMILES,  $EC_{50}$  values and raw data for all 114 compounds are given in the [Supplementary material](#). Of these, two were known anti-malarials (quinidine and amodiaquine) selected as positive control. In addition, 31 compounds (six from Workflow 1 and 28 from Workflow 2, three were in common) were already present in the primary HTS and confirmatory screen provided by the TDT challenge, as such molecules were not explicitly removed from the eMolecules catalog before the virtual screen ([Supplementary Table S1](#)). One of these compounds, SJ000154494 ([Figure 3](#),  $EC_{50}$  = 0.44  $\mu$ M as measured in this study) was found inactive in the previous primary screen and confirmatory screen<sup>23</sup>, which was likely a false negative in the latter screen because dose-response testing immediately following the primary screen was done using compounds from stock solutions ranging in age, whereas the current experiment was performed on fresh powder.

The results for the remaining 81 new compounds and the two known anti-malarials are listed in [Table 5](#). A list of all 114 compounds, including SMILES is provided as a separate file in the [Supplementary material](#). Partially active or single-point active molecules were counted as inactives. As the list of 1000 compounds in Workflow 1 was randomly selected from the top 10,000 ranked compounds in the eMolecules database, the ranks in the latter list are also reported in [Table 5](#). From the nine molecules proposed by Workflow 2, only two were not in the top 10,000 list from Workflow 1, indicating that the two approaches pick generally similar features but do not score them in the same manner. Of

the 81 new compounds, 46 were found to be active, resulting in an overall hit rate of 57%. In more detail, Workflow 1 gave a hit rate of 52% and Workflow 2 a hit rate of 100%. Due to the small number of compounds tested, we cannot judge if this difference in hit rate is significant. As the TDT initiative relies on contributions of compounds, a more systematic assessment is outside the scope of this effort. Interestingly, the most active compounds were ranked rather low in the top-1000 list of Workflow 2 and the top-10,000 list of Workflow 1 compared to the other molecules tested, which emphasizes again that it is important in ligand-based VS to pick the compounds for follow-up testing relatively broadly from the top fraction.

For Workflow 1, six of the 73 new compounds were tested previously in anti-malaria activity assays found in ChEMBL-NTD (<https://www.ebi.ac.uk/chemblntd/>) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>) and three of them were found to be active. Three main scaffolds covered 25 of the 73 compounds: thiazolidin-4-one-type, 8-hydroxyquinoline-type, and aminopyrimidine-type scaffolds ([Table 6](#)). The compounds with the thiazolidin-4-one-type scaffold were the largest group. The scaffold can be seen as a variation of compound SJ000154494 ([Figure 3](#)), but the compounds in this group were mostly inactive. In addition, the scaffold may be a potential PAINS substructure due to its similarity with rhodanine, although it is currently not part of the filters<sup>28</sup>. The 8-hydroxyquinoline scaffold is a phenolic Mannich base, which is a PAINS substructure. The most interesting scaffold is the aminopyrimidine-type with a second *N*-alkyl substituent instead of a known *N*-aryl substituent. The most active compound of this series, SJ000866807, exhibits a good ligand efficiency with an  $EC_{50}$  of 0.2  $\mu$ M and a molecular weight of only 266 g/mol. From this series of compounds only one (SJ000866811) was listed in PubChem, but this was in an assay for anti-cancer activity (AID 743276). However, similar compounds were previously reported in the Novartis-GNF Malaria Box<sup>40</sup> ([Figure 4](#)).

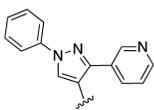
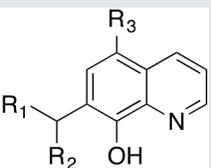
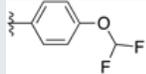
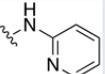
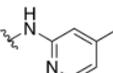
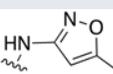
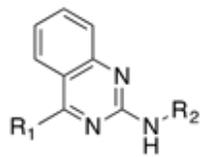
**Table 5. Results from the follow-up assay for 83 compounds.** The columns are as follows: EC<sub>50</sub> values, the final scores (active or inactive), and the ranks in the Workflows 1 and 2. Partially active or single-point active compounds were considered inactives (marked by italic font). ChEMBL-NTD datasets: Novartis-GNF Malaria Box (N)<sup>40</sup>, St. Jude Children's Research Hospital Dataset (J)<sup>24</sup>, GSK TCAMS (G)<sup>41</sup>, DNDi HAT set (D). Compounds marked with (P) were tested in PubChem assays.

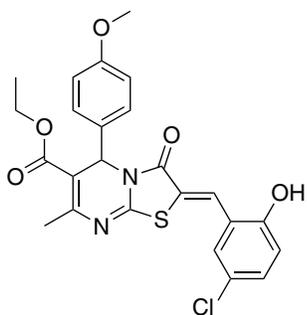
Identifier	EC <sub>50</sub> [μM]	Score	Rank (1000) Workflow 1	Rank (top 10'000) Workflow 1	Rank (top 1000) Workflow 2	Known Datasets
SJ000110703	0.025	Active		3907	853	Amodiaquine
SJ000285572	0.060	Active	867	6589		Quinidine
SJ000866784	0.099	Active		4544	931	
SJ000866752	0.14	Active		3108	826	
SJ000866753	0.18	Active	725	5240		
SJ000866807	0.20	Active	613	4337		
SJ000361770	0.28	Active		3394	952	
SJ000866781	0.29	Active	476	3299		
SJ000866764	0.39	Active		2174	720	N, P (active)
SJ000866760	0.72	Active	300	1739		
SJ000866797	0.76	Active		-	868	P (anti-malaria: AID504832, AID504834) (active)
SJ000866778	0.77	Active		-	984	
SJ000866810	0.84	Active		974	100	N, J, G (active)
SJ000866811	0.92	Active	829	6197		P (not anti-malaria)
SJ000866815	0.98	Active		9752	569	P (anti-malaria: AID504382) (active)
SJ000866767	1.1	Active	597	4262		
SJ000866780	1.2	Active	171	688		
SJ000866773	1.3	Active	697	5138		
SJ000866792	1.5	Active	950	7129		
SJ000866800	1.9	Active	714	5205		N (active)
SJ000377329	2.0	Active	807	6068		
SJ000866786	2.3	Active	784	5832		
SJ000364456	2.4	Active	808	6073		
SJ000866779	3.2	Active	456	3069		D (inactive)
SJ000866794	3.2	Active	559	3935		
SJ000866757	3.3	Active	901	6813		
SJ000866813	4.1	Active	509	3613		
SJ000866809	4.3	Active	403	2603		
SJ000377299	4.4	Active	844	6318		
SJ000866777	4.6	Active	584	4159		
SJ000866750	5.4	Active	798	6016		
SJ000866789	7.8	Active	830	6198		
SJ000866790	8.2	Active	409	2624		
SJ000866755	8.4	Active	789	5923		
SJ000399327	9.2	Active	617	4383		P (anti-malaria: AID504832, AID504834) (active)
SJ000866806	9.2	Active	785	5850		
SJ000866759	9.3	Active	927	7004		
SJ000866747	9.5	Active	610	4303		
SJ000866799	9.5	Active	546	3852	989	D (active)

Identifier	EC <sub>50</sub> [ $\mu$ M]	Score	Rank (1000) Workflow 1	Rank (top 10'000) Workflow 1	Rank (top 1000) Workflow 2	Known Datasets
SJ000866766	9.7	Active	510	3614		
SJ000866749	10.0	Active	561	3942		
SJ000866793	10.0	Active	590	4191		
SJ000866768	12.0	Active	444	2939		
SJ000866762	12.0	Active	474	3282		
SJ000866788	14.0	Active	238	1166		
SJ000866798	14.0	Active	852	6416		
SJ000420481	17.0	Active	484	3378		
SJ000866776	18.0	Active	35	61		
SJ000866769	3.7	Inactive	768	5649		
SJ000866796	4.6	Inactive	521	3684		
SJ000866804	6.1	Inactive	264	1366		
SJ000866765		Inactive	18	31		
SJ000866771		Inactive	600	4272		
SJ000866783	7.2	Inactive	747	5414		P (anti-malaria: AID504832, AID504834) (inactive)
SJ000866802	7.9	Inactive	401	2598		
SJ000866808	11.0	Inactive	570	4051		
SJ000866748	11.0	Inactive	850	6407		
SJ000866785	19.0	Inactive	711	5202		
SJ000866751	6.0	Inactive	197	880		
<b>SJ000389261</b>	6.0	Inactive	998	7634		P (anti-malaria: AID504832, AID504834) (inactive)
SJ000866758	8.8	Inactive	859	6525		
SJ000866746	15.0	Inactive	816	6110		
SJ000866782		Inactive	14	23		
SJ000866803		Inactive	360	2269		
SJ000866805		Inactive	379	2468		
SJ000388303		Inactive	411	2630		
SJ000866770		Inactive	437	2879		
SJ000866801		Inactive	504	3588		
SJ000866772		Inactive	506	3600		
SJ000866775		Inactive	563	3948		
SJ000866763		Inactive	618	4385		
SJ000866761		Inactive	620	4405		
SJ000866745		Inactive	704	5167		
SJ000866791		Inactive	741	5376		
SJ000866812		Inactive	779	5792		
SJ000866795		Inactive	952	7149		
SJ000866756		Inactive	191	852		
SJ000866754		Inactive	250	1257		
SJ000866814		Inactive	404	2606		
SJ000394036		Inactive	457	3073		
SJ000866774		Inactive	547	3858		
SJ000866787		Inactive	695	5124		
SJ000391199		Inactive	828	6194		

**Table 6. The three main scaffolds present in the 73 compounds predicted by Workflow 1.**  
ChEMBL-NTD datasets: Novartis-GNF Malaria Box (N)<sup>40</sup>, St. Jude Children's Research Hospital Dataset (J)<sup>24</sup>, GSK TCAMS (G)<sup>41</sup>, DNDi HAT set (D). Compounds marked with (P) were tested in PubChem assays.

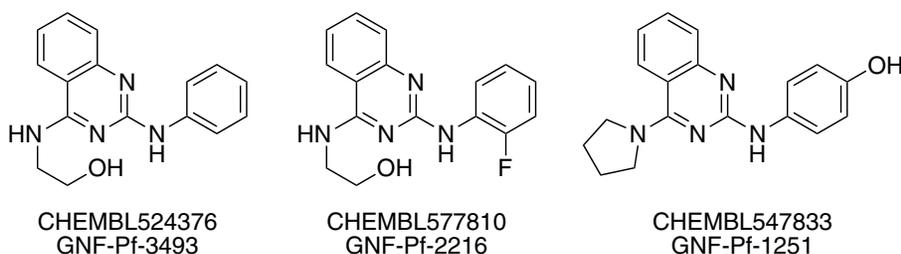
Identifier	R1	R2	R3	EC <sub>50</sub> [μM]	Known Datasets
SJ000388303	H		H	-	
SJ000391199	H		H	-	
SJ000389261	H		H	6.0	P (anti-malaria: AID504832, AID504834)
SJ000394036	H		H	-	
SJ000866774	H		H	-	
SJ000866791	H		H	-	
SJ000866759	H		H	9.3	
SJ000866776	H		H	18.0	
SJ000866756			H	-	
SJ000866814	Phenyl-		Cyano-	-	
SJ000866809			Cyano-	4.3	
SJ000866805			Cyano-	-	

SJ000866804			Cyano-	6.1	
SJ000866802			Cyano-	7.9	
SJ000866801			Cyano-	-	
					
Identifier	R1	R2	R3	EC <sub>50</sub> [μM]	Known Datasets
SJ000866799			H	9.5	D
SJ000866771			H	-	
SJ000866779			H	3.2	D
SJ000866777			H	4.6	
SJ000866800			H	1.9	N
					
Identifier	R1	R2	EC <sub>50</sub> [μM]	Known Datasets	
SJ000866807			0.20		
SJ000866760			0.72		
SJ000866811			0.92	P (not anti-malaria)	
SJ000377329			2.0		
SJ000377299			4.4		

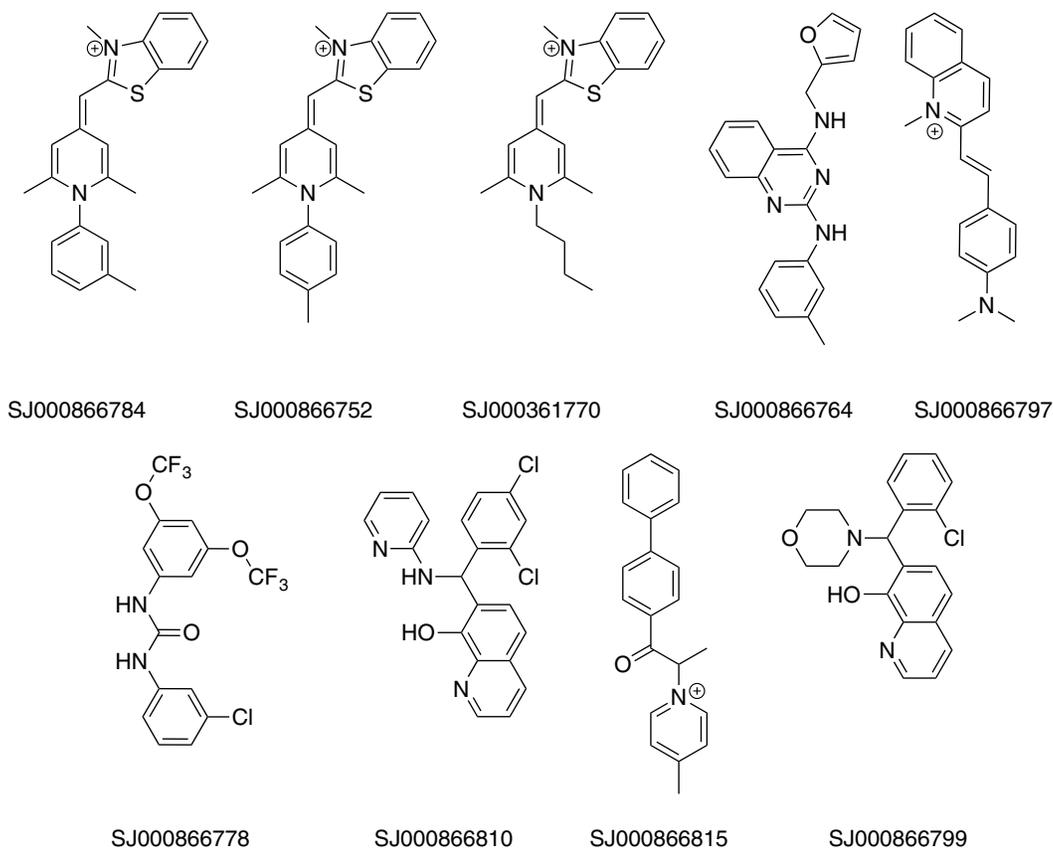


**Figure 3.** Compound **SJ000154494** ( $EC_{50} = 0.44 \mu\text{M}$  as measured in this study).

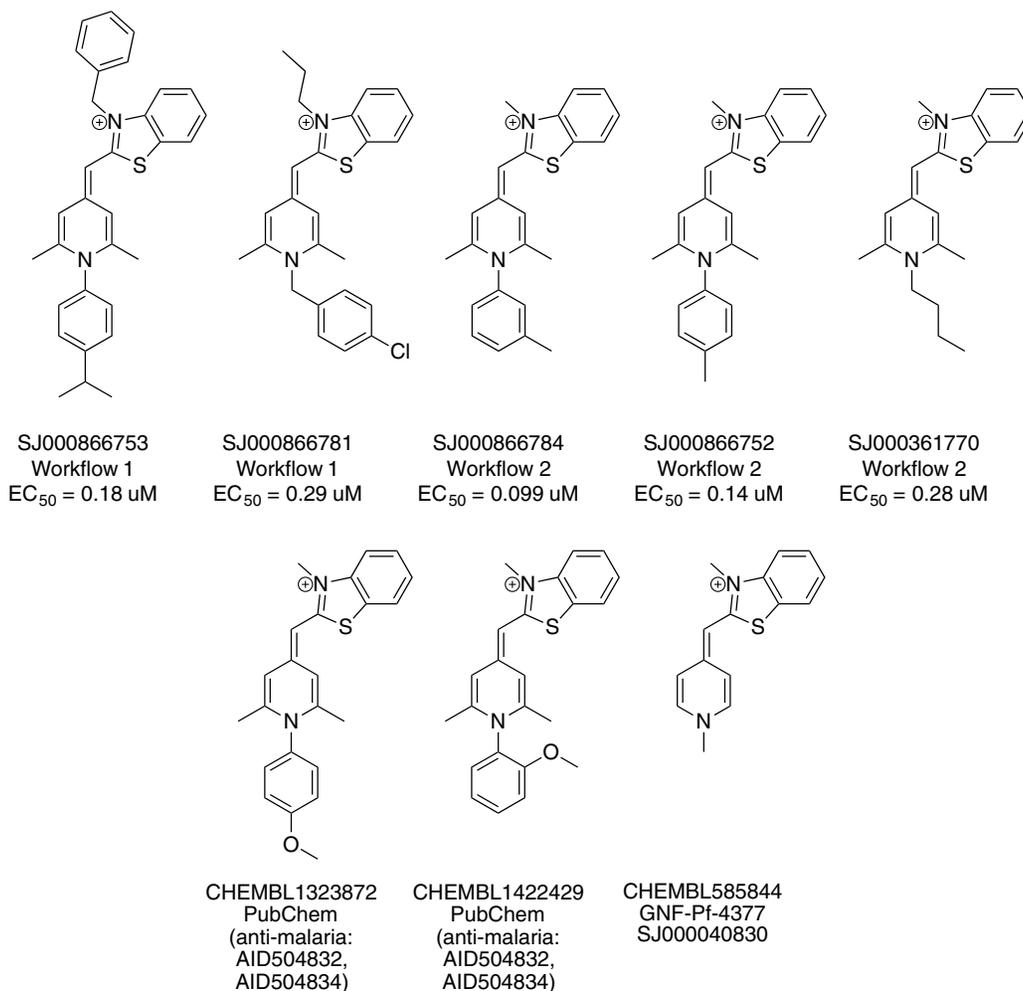
The nine new compounds proposed by Workflow 2 are shown in **Figure 5**. Five of them had been tested active previously in one of the ChEMBL-NTD assays or in PubChem assays for anti-malaria activity. Two compounds (SJ000866810 and SJ000866799) have the same 8-hydroxyquinoline-type scaffold as in Workflow 1, and one compound (SJ000866764) has a similar aminopyrimidine-type scaffold. Among the most active compounds predicted by both Workflows was a series of molecules with a benzothiazole scaffold (**Figure 6**). Compounds with a similar scaffold were tested previously in PubChem assays for anti-malaria activity or are part of the ChEMBL-NTD datasets. Compound **SJ000040830** showed also high anti-leishmanial activity<sup>23</sup>. There may be, however, potential PAINS issues with this scaffold, although not covered by the



**Figure 4.** Compounds from the Novartis-GNF Malaria Box<sup>40</sup>, with an aminopyrimidine-type scaffold. These compounds are similar to the group of compounds predicted by Workflow 1 with the same scaffold (**Table 6**).



**Figure 5.** Nine compounds proposed by Workflow 2. The molecules are ordered by decreasing activity.



**Figure 6. Compounds with a benzothiazole scaffold.** (Top): Compounds predicted by Workflow 1 and Workflow 2. (Bottom): Compounds that are actives from PubChem, Novartis-GNF Malaria Box<sup>40</sup> and St. Jude Children's Research Hospital<sup>24</sup>.

current PAINS filters, as the extended  $\pi$ -system may act as Michael-like acceptor.

## Conclusions

The use of ligand-based VS based on results from a primary HTS to select new, potentially active compounds for testing is a common task in drug discovery. Here, we presented two detailed Workflows using open-source tools for educational purposes, and report the application of these Workflows for the identification of anti-malarial compounds as part of the 2014 TDT challenge. Information from a previous primary HTS performed at the St. Jude Children's Research Hospital (and a confirmatory screen in case of Workflow 2) was used for training. Of the 2000 compounds proposed by the Workflows, 114 were selected for follow-up testing based on availability. Excluding the two known anti-malarials quinidine and amodiaquine and the 31 compounds already present in the primary screen, 46 out of 81 new compounds

were found to be active, which corresponds to a high hit rate of 57% and shows that the machine-learning methods in the presented Workflows both successfully identified scaffolds with anti-malaria activity. There was a good agreement between the two Workflows in the general scaffolds that were identified, even though the exact compounds and rankings were not the same. The most interesting group of compounds in the tested set contains an aminopyrimidine-type scaffold with a second *N*-alkyl substituent instead of a known *N*-aryl substituent. In particular, the most active compound SJ000866807 of this series shows good ligand efficiency.

## Data and software availability

The tutorials are available on the TDT website (<http://www.tdt-project.org/2012-competition--tutorials.html>) and on GitHub (<https://github.com/sriniker/TDT-tutorial-2014> and <https://github.com/sdvillal/tdt-malaria-followup>). Both tutorials use only freely

available software as specified above. The data from the primary HTS and confirmatory dose-response assay used in the TDT competition are available on the TDT website (<http://www.tdtproject.org/challenge-1---malaria-hts.html>) and are also deposited in ChEMBL, as part of the Neglected Tropical Diseases set (ChEMBL-NTD). The identifiers, SMILES, EC<sub>50</sub> values and raw data for the held-out test set<sup>25</sup>, as well as for the 114 compounds tested in this study, are given in the [Supplementary material](#).

### Author contributions

SR and GL have created and applied Workflow 1. FM and SV have created and applied workflow 2. AS and JM have performed the follow-up assay. JJ and PW are members of the TDT steering committee and have organized the acquisition of chemical substances from vendors for testing in the follow-up assay.

### Supplementary material

Supplementary Table S1: Identifiers, EC<sub>50</sub> values, final scores and ranks in the Workflows 1 and 2 for the 31 tested compounds that were part of the primary HTS screen.

[Click here to access the data.](#)

Supplementary Table S2: Identifiers, SMILES, EC<sub>50</sub> values and raw data for the 1056 molecules in the external held-out test set.

[Click here to access the data.](#)

Supplementary Table S3: Identifiers, SMILES, EC<sub>50</sub> values and raw data for the 114 molecules tested in this study.

[Click here to access the data.](#)

### Competing interests

The authors declare no competing financial interests.

### Grant information

SR thanks the Novartis Institutes for BioMedical Research education office for a Presidential Postdoctoral Fellowship.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

### Acknowledgements

The authors thank eMolecules as TDT partner in the compound acquisition, and ChemBridge and Enamine for providing compounds. The authors also thank the other sponsors of TDT (<http://www.tdtproject.org/partners-and-sponsors.html>) and the TDT steering committee (<http://www.tdtproject.org/steering-committee.html>).

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# Open Peer Review

Current Referee Status:  

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Version 1

Referee Report 16 August 2017

doi:10.5256/f1000research.12868.r24675



**Matthew P. Baumgartner** 

Eli Lilly and Company, Windlesham, UK

The authors report on their participation in the 2014 Teach-Discover-Treat (TDT) initiative. The goal of the TDT is to encourage the creation of practical tutorials for computational chemistry. The authors present the two workflow tutorials that they developed for Challenge 1 of the competition. The challenge involved three tasks: analyzing single-point phenotypic HTS results and follow-up dose-response data for a subset of the compounds, building a predictive model of the anti-malaria activity and using that predictive model to select compounds from a set of commercially available compounds for prospective testing. The first workflow presented by the authors only used the HTS data for its predictions and the second used both the HTS and dose-response data.

Overall I think that the paper is a thorough and easy-to-follow description of the methods and results of the two workflows, but there a few items that I feel require revisions.

Page 5, a brief description of what "heterogeneous classifier fusion" is would be appreciated

Page 6. The authors should list the total number of features that they use as descriptors in workflow 2.

Page 6. When building the random forests and extremely randomized trees of varying sizes, the ensembles of trees with 6000 trees (the largest number tested) were shown to perform best. The authors should explain why they did not try a higher number of trees.

Page 6. In the "Task 2..." paragraph. The authors should state what the resulting linear combination of the models was. The ratio would be interesting to know.

Page 8, in the paragraph starting "The results for the remaining...". It states in the text that there were 9 compounds predicted by workflow 2 that tested, but in Table 5, there are 10 compounds from workflow 2 listed. This should be corrected or clarified.

Page 8 and Table 5. As the compounds from Workflow 1 were selected randomly due to an error, is it meaningful to list their rankings at all?

**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** computational chemistry

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 02 August 2017

doi:[10.5256/f1000research.12868.r24285](https://doi.org/10.5256/f1000research.12868.r24285)



**David Ryan Koes** 

Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA, USA

The report describes the two winning ligand-based virtual screening methods of the 2014 Teach-Discover-Treat exercise. Both workflows and the supporting data are available in their entirety online (behind a request for the user's email address) and are adequately described in the manuscript. The report is of general interest to the community and a useful resource to practitioners in ligand-based drug discovery.

I have a few minor suggestions for strengthening the manuscript.

Page 4. A few sentences describing "heterogeneous classifier fusion" would be appreciated.

Page 5. Descriptors. I would be interested in knowing the number of bits (i.e. unique ECFP/FCFP fragments) required to represent the full dataset (that is, the number of features in the input, which I suspect is actually larger than the number of examples?).

Page 5. Task 2. The weights for the two models found by the linear regression would be interesting to report (is one model favored more heavily than the other?).

Table 4. This would be a bit more informative if variance was reported as well.

Figure 2. It isn't clear to me exactly what this is reporting. Is this the distribution of all possible pairs between the two sets? Please clarify.

Table 5. My understanding is that the Rank (1000) numbers are essentially meaningless as the compounds were (accidentally) randomly selected. Can the corrected top 1000 ranks be provided as well (or instead) and clearly labeled as such (realizing that not all compounds will have such a rank).

It's also hard to get a sense of enrichment from these numbers since only 114 compounds were tested but the ranks have a much larger span. For example, the workflow 2 active compounds have poor ranks (>500), but this is misleading since there were no highly ranked (novel) compounds tested. I would really appreciate some visualization of enrichment relative to ranking (e.g. ROC curve) for 114/81 compounds tested for workflow 1.

**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** computational drug discovery

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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