**Identification of novel inhibitors for the tyrosyl-DNA-phosphodiesterase 1 (Tdp1) mutant SCAN1 using virtual screening**

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**Abstract**

Spinocerebellar ataxia syndrome with axonal neuropathy (SCAN1) is a debilitating neurological disease that is caused by the mutation the Tyrosyl-DNA phosphodiesterase 1 (TDP1) DNA repair enzyme. The crucial His493 in TDP1’s binding site is replaced with an arginine amino acid residue rendering the enzyme dysfunctional. A virtual screen was performed against the homology model of SCAN1 and seventeen compounds were identified and tested in a novel SCAN1 specific biochemical assay. Six compounds showed activity with IC50 values between 3.5 and 25.1 µM. The most active ligand **5** (3.5 ± 2.3 µM) is a dicoumarin followed by a close structural analogue **6** at 6.0 µM. A less potent series of β-carbolines (**14** and **15**) was found with potency in the mid-teens. According to molecular modelling an excellent fit for the active ligands into the binding pocket is predicted. Data on inhibitors of the mutant form of TDP1 has not been reported previously to our knowledge. The virtual hits were also tested for wild type TDP1 activity and all six SCAN1 inhibitors are potent for the former, *e.g.*, ligand **5** has a measured IC50 at 99 ± 44 nM. The active ligands are mostly non-toxic to cancer cell lines A-549, T98G and MCF-7 as well as the immortalized WI-38 human fetal lung cells. Furthermore, ligands **5** and **7**, show promising synergy in conjunction with topotecan, a clinically used topoisomerase 1 anticancer drug. The active ligands **5**, **7**, **14** and **15** have a good balance of the physicochemical properties required for oral bioavailability making the excellent candidates for further development.

**1. Introduction**

The development of DNA repair enzymes inhibitors is currently an important goal. The design of drugs halting DNA repair pathways can provide new specific and less toxic treatments for oncological, cardiovascular, and neurodegenerative diseases. These pathologies are often associated with alterations in DNA repair genes structure and aberrations in DNA repair protein expression.[[1]](#endnote-1),[[2]](#endnote-2),[[3]](#endnote-3)

Tyrosyl-DNA phosphodiesterase 1 (TDP1) plays a key role in the removal of damage caused by topoisomerase 1 (Top1) poisons such as topotecan and irinotecan,[[4]](#endnote-4) which are widely used in clinical practice. This makes TDP1 a promising target to enhance anticancer treatment in conjunction with established therapies based on DNA damage. A number of studies support the hypothesis that TDP1 is responsible for drug resistance in some cancer types.[[5]](#endnote-5) On the one hand, TDP1-/- mice and cell lines with SCAN1 (spinocerebellar ataxia syndrome with axonal neuropathy) mutation, that reduces *in vitro* catalytic activity compared to the wild type enzyme, are hypersensitive to camptothecin,[[6]](#endnote-6),[[7]](#endnote-7),[[8]](#endnote-8) a Top1 poison. Antibiotic monocyclin enhances the antimetastatic effect of irinotecan by reducing expression of TDP1 as well as increasing the life span of experimental animals.[[9]](#endnote-9) On the other hand, cell lines with enhance TDP1 expression are less prone to DNA damage when dosed with camptothecin.[[10]](#endnote-10),[[11]](#endnote-11) Moreover, TDP1 overexpression in colorectal cancer cells leads to poor response to irinotecan therapy.[[12]](#endnote-12) These findings suggest that a combination of DNA damaging drugs and TDP1 inhibitors can significantly improve the effectiveness of chemotherapy.

The literature reports on a number of TDP1 inhibitors, with potency varying from submicromolar to millimolar values.[[13]](#endnote-13),[[14]](#endnote-14) Previously, we found effective TDP1 inhibitors based on usnic acid,[[15]](#endnote-15),[[16]](#endnote-16),[[17]](#endnote-17),[[18]](#endnote-18) aminoadamantanes,[[19]](#endnote-19),[[20]](#endnote-20) benzopentathiepines,[[21]](#endnote-21) bile acids,[[22]](#endnote-22) chromenes,[[23]](#endnote-23) thieno[2,3-b]pyridines,[[24]](#endnote-24) and coumarin derivatives.[[25]](#endnote-25) The combination of some of these inhibitors with camptothecin or its derivative topotecan showed synergistic effect on tumor cell lines.15,16,17,25 Recently, the usnic acid hydrazinothiazole derivative **20d** was shown to significantly increase the antitumor and antimetastatic effect of topotecan in mice. The combined effect of topotecan and **20d** reduced the tumor weight by 57.6% compared to the control and metastases by an order of magnitude compared to the effect of topotecan only.17

TDP1 is a member of phospholipase D superfamily[[26]](#endnote-26) and catalyzes the cleavage of phosphodiester bond between the 3’-phosphate with the aid of a tyrosine residue (Tyr723 of the human enzyme) through two sequential SN2 reactions. DNA-Top1 binds to the substrate-binding pocket where the 3’-phoshate is stabilized by hydrogen bonds with the Lys265 and Lys495 residues. The first step involves nucleophilic attack of the 3’-phosphate by the Nε2 atom of His263 (Fig. 1A) The product of this reaction is the TDP1-DNA covalent cleavage complex. In the second step, the complex is hydrolyzed via nucleophilic attack of a His493-activated water molecule (Fig. 1B). The final product of the catalytic reaction is a 3’-phosphate DNA strand (Fig. 1C).[[27]](#endnote-27),[[28]](#endnote-28)

Изображение выглядит как текст, карта

Автоматически созданное описание

**Fig. 1.** Proposed reaction mechanism for human TDP1. The first step of the reaction involves the nucleophilic attack of the phosphodiester-containing substrate by the imidazole Nε2 atom of His263 (A). His493 donates a proton to the tyrosyl residue of the leaving group. The phosphohistidine intermediate occurs. The next step of the reaction involves a second nucleophilic attack by a water molecule activated by His493 (B), generating final 3′-phosphate product and free Tdp1 (C).The SCAN1 mutation (H493R) leads to the accumulation of the Tdp1-DNA intermediate and a defect in Tdp1 turnover rate (D).[[29]](#endnote-29)

SCAN1 is a natural mutant of TDP1 where His493 is replaced with Arg493 in the binding pocket. The mutation changes the geometry of the enzyme active site, which increases the distance between catalytic amino acids Arg493 and His263. Therefore, the second stage of hydrolysis of the covalent cleavage complex does not occur and the enzyme remains covalently bound to DNA (Fig. 1D). Potentially SCAN1-DNA adduct can be removed by “noncatalytic” hydrolysis albeit much slower thancatalytically.[[30]](#endnote-30) This mutation leads to a severe neurodegenerative disease spinocerebellar ataxia syndrome with axonal neuropathy (SCAN1).[[31]](#endnote-31) The exact molecular mechanisms leading to the SCAN1 disease is still unclear. However, despite TDP1 being a DNA repair enzyme, the frequency of oncological or other diseases associated with defects in the DNA repair system is not increased in patients with SCAN1.31 Probably, the pathology is caused by the accumulation of the SCAN1-DNA covalent cleavage complexes formed.[[32]](#endnote-32) It should also be noticed that this mutation leads to the mitochondrial dysfunction which presumably contributes to the pathology of SCAN1.[[33]](#endnote-33) Therefore, suppression of SCAN1 activity could potentially improve the SCAN1 patients’ condition and prevent the progression of the disease. It should be noted that the search SCAN1 inhibitors has not yet been conducted.

In the present research, we conducted a virtual screening of a library of natural products and their derivatives for their ability to inhibit the SCAN-TDP1 formation. Coumarin and β-carboline based ligands were identified, their effect on the TDP1 and SCAN1 enzymes was determined as well as their cytotoxicity against different cell lines. Furthermore, some of the coumarin and β-carboline derivatives showed synergistic effect with topotecan on the tumor cell lines A-549 and T98G.

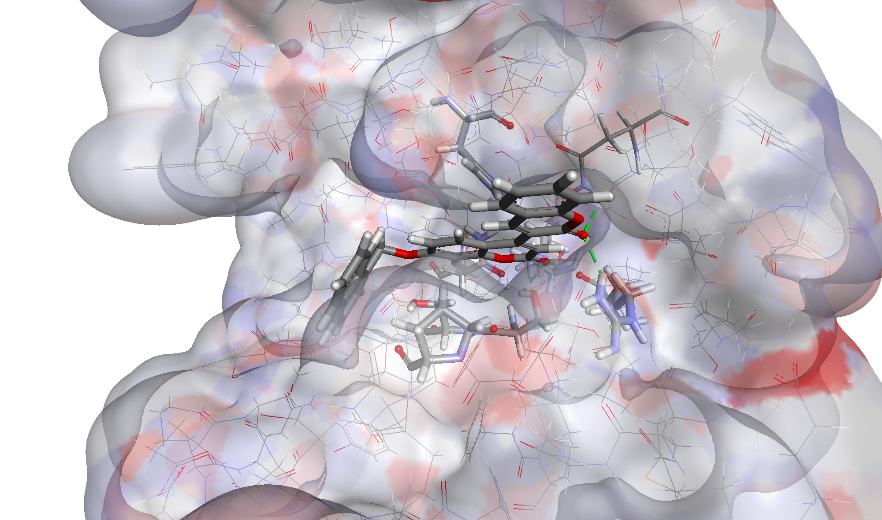
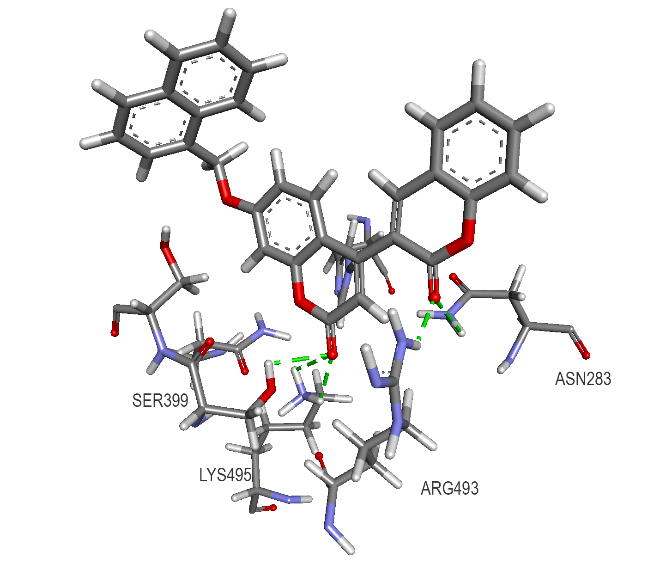
**2. Results and discussion**

**2.1. Virtual screening**

The virtual screening protocol used has been previously successfully applied to a host of different bio-molecular targets, *e.g.*, phospholipase C – γ,[[34]](#endnote-34) autophagy,[[35]](#endnote-35) tyrosyl-DNA phosphodiesterase I22,25 and heat shock protein 90.[[36]](#endnote-36)

The TDP1 1MU727 protein structure was edited using the Scigress software suite to make the histidine to arginine (H493R) mutation. The imidazole side chain of His493 was modified in to the aliphatic guanidine moiety representing Arg. The remaining amino acids were locked in position and the PM3 semi-emprical method[[37]](#endnote-37) was used to optimize the geometry of Arg493. The screen was conducted with 9.2×103 molecules from the InterBioScreen natural product collection. The GoldScore (GS),[[38]](#endnote-38) ChemScore (CS),[[39]](#endnote-39),[[40]](#endnote-40) Piecewise Linear Potential (ChemPLP)[[41]](#endnote-41) and Astex Statistical Potential (ASP)[[42]](#endnote-42) scoring functions were used to assess the binding of the ligands. The virtual screen was done in two phases using relatively low screening efficiency (30%) weeding out compounds with predicted low binding affinity followed by a more robust search for the remaining ligands. First, all ligands were screened and those with no or weak predicted hydrogen bonding (<1) were eliminated as well as those with low predicted binding energies (GS<52, CS<24, ChemPLP<60 and ASP<30). This left 380 candidates, which were screened again with high search efficiency (100%) and again were eliminated based on their binding energies (GS<57, CS<25, ChemPLP<63 and ASP<26) and poor hydrogen bonding (<1) resulting in 144 candidates.

Compounds were chosen based on favourable hydrogen bonding and hydrophobic contacts. Based on docking studies the key hydrogen bonding interaction is with the histidine residues (493 & 263) and due to the H493R mutation the scope of potential hydrogen bonding interactions was opened. Compounds that had predicted binding to other amino acids were also included. Histidine (263), asparagine (283, 288, 516), serine (399,400, 518), lysine (295) and tyrosine (204) were all included as acceptable for hydrogen bonding interactions. The criteria for hydrophobic contacts remained the same as previous TDP1 screens.22,25 Occupation of either the hydrophobic cleft situated to the left hand side or the hydrophobic pocket on the right hand side as shown in Figure 2 were deemed acceptable. Compounds that scored well, gave a consensus pose (similar/same poses predicted by the four scoring funcitons) and met the above criteria were selected for testing resulting in seventeen compounds (see Table 1).

**A****B**

**Fig. 2** (A) The docked configuration of **8** to the binding site of SCAN1 using the ChemPLP scoring function. The protein surface is rendered, blue depicts positive areas surface, and red depicts negative areas on the surface. The hydrophobic cleft is shown to the left and the hydrophobic pocket to the right. (B) The docked configuration of **8** to the binding site of SCAN1using the ChemPLP scoring function. Predicted hydrogen bonding to Lys495, Arg493 Asn283 and Ser399 was seen. The hydrogen bonding is depicted as green dashed lines and are in the range of 1.8 to 2.2 Å.

**2.2. TDP1 and SCAN1 activity**

# Previously, we designed real-time oligonucleotide biosensor based on the capability of TDP1 to remove fluorophore quenchers from the 3’-end of DNA. The substrate is a 16-mer single-stranded oligonucleotide containing both a 5’-FAM fluorophore donor and a quenching 3’-BHQ moiety.21

This assay was used to measure the TDP1 activity of the virtual hits. The results for the coumarins, β-carbolines, psoralens and one peptide compound are presented in Table 1. The reaction between TDP1 and the DNA substrate occurs through two sequential steps: the cleavage of the bond between DNA and 3’-adduct (tyrosine moiety in the case of Top1/DNA complex or BHQ1 in the case of DNA biosensor) with the formation of TDP1-DNA covalent complex and its subsequent splitting. SCAN1 can perform only the first step of the reaction so BHQ1 is removed from 3’-end of DNA but it remains covalently bound to SCAN1. This means that DNA-biosensor can also be used to determine the rate of the BHQ1 removal reaction performed by SCAN1. It should be taken into account that each wild-type enzyme is able to perform several different catalytic cycles, while the SCAN1 molecule can perform only one; therefore, the rate of increase in fluorescence intensity is significantly different. In addition, it is important to mention that SCAN1 does not act like classical enzyme as it is consumed by the reaction. This is why the conditions of the reactions catalyzed by TDP1 and SCAN1 are different and their IC50 values (concentration leading to 50% inhibition of activity) obtained for the same compounds are not directly comparable. All the results are given in Table 1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Class of inhibitor | Number of compound | Chemical structure | IC50 (SCAN1), μM | IC50 (TDP1), μM |
| Coumarin | **1** |  | > 100 | 3.5±0.3 |
| **2** |  | > 100 | 5.6±1.9 |
| **3** |  | > 100 | 12.9±3.0 |
| **4** |  | > 100 | > 100 |
| Dicoumarin | **5** |  | 3.5±2.3 | 0.099±0.044 |
| **6** |  | 13.9±5.6 | 0.28±0.03 |
| **7** |  | 6.0±1.5 | 0.36±0.19 |
| **8** |  | > 100 | 0.27±0.19 |
| **9** |  | > 100 | 7.6±1.3 |
| Psoralene | **10** |  | 25.1±13.7 | 1.06±0.56 |
| **11** |  | > 100 | 0.85±0.5 |
| **12** |  | > 100 | 15.4±4.5 |
| **13** |  | > 100 | > 100 |
| β-carboline | **14** |  | 17.6±6.1 | 0.98±0.6 |
| **15** |  | 14.1±1.9 | 1.3±0.1 |
| **16** |  | > 100 | 3.1±0.8 |
| Peptide | **17** |  | > 100 | > 100 |

**Table 1.** The IC50 values (concentration leading to 50% inhibition of activity) of the virtual hits for both TDP1 and SCAN1.

Generally, dicoumarins (**5**-**9**) and β-carbolines (**14**-**16**) show better inhibitory activity against both TDP1 and SCAN1 enzymes than monocoumarins (**1**-**4**) and psoralens (**10**-**13**). The peptide **17** was inactive against both enzimes.

The most effective inhibitors of TDP1 are dicoumarins **5, 6**, **7** with IC50 values between 3.5 and 13.9 μM. They contain chlorine substituents as well as dicoumarin **8** contains a bulky naphthalene moiety. At the same time for dicoumarin **9** with rather small hydroxyacetone residue a decrease of the IC50 value by almost two orders of magnitude was observed as compared to **5**. The similar situation is observed for β-carbolines. In this series (**14**, **15** and **16**)inhibitory activity decreases as the size of the substituent decreases. The psoralen derivatives **12** and **13** have no, or very limited activity, whereas the compounds **10** and **11** with bulky chlorobenzene and fluorobenzene moieties are the active inhibitors against TDP1. Most monocoumarins showed moderate inhibitory activity (IC50 = 3.5-12.9 μM) against TDP1.

To confirm the IC50 values obtained with the fluorescent method for TDP1 enzyme we determined the IC50 value of compound **10** using the independent method of electrophoretic separation of reaction products under denaturing conditions in PAAG (Supplementary, Fig. S1). The values obtained by fluorescent and gel-based methods differed insignificantly: 1.06 µM and 1.75 µM, respectively (Supplementary Fig. S2).

The most effective inhibitors against SCAN1 are the same as those against TDP1 dicoumarins **5**, **6** and **7**. There are also some SCAN1 inhibitors among the psorales (**10**) and β-carbolines (**14**, **15**) efficiently suppressing TDP1 activity.

It is important to notice that some effective inhibitors of TDP1 with low IC50 values (coumarins **1**-**3** and **8**, psoralens **11**-**13**, carboline **16**) do not inhibit SCAN1. As it was mentioned before, the reaction catalyzed by TDP1 occurs via two sequential steps: formation of the TDP1-DNA covalent cleavage complex in the first step and its cleavage on the second step (Fig.1A-C). As for SCAN1 catalysis, it involves only the first step of reaction, *i.e.* the formation of a covalent complex, whereas the second step (hydrolysis) does not occur (Fig. 1D). It can be assumed that the effective TDP1 inhibitors incapable to inhibit SCAN1 suppress the second step of the reaction, which is missing in SCAN1. At the same time compounds inhibiting both enzymes suppress the first step of the reaction preventing DNA binding or DNA-TDP1 covalent complex formation.

Compounds suppressing the second step of reaction (incapable to inhibit SCAN1) prevent the hydrolysis of DNA-TDP1cc complex so they act through its stabilization. It can lead to the accumulation of these complexes by the same way as in SCAN1 disease. Therefore, the use of such compounds in antitumor therapy can cause side effects in the nervous system similar to that for SCAN1 patients. Both types of inhibitors are suitable for creating drugs for adjuvant cancer therapy, but the possible neurotoxicity of compounds that do not affect the activity of the SCAN1 should be taken into account. As for the treatment of SCAN1 patients only inhibitors suppressing the first step of reaction can be used.

When the numbers from the four scoring functions used are compared to their SCAN1 IC50 values (see Table S4 in the Supplementary Information), only a general trend can be discerned. The prediction power of scoring functions is often good but there is still room for improved. (Ref. Wang, *Phys.Chem.Chem.Phys.,*2016, 18, 12964 DOI: 10.1039/c6cp01555g) Furthermore, the docking scaffold of SCAN1 used is a homology model based on TDP1 making a robust correlation less likely.

**2.3. Cell growth and viability**

To measure the cytotoxic effect of the TDP1 and SCAN1 inhibitors three cancer cell lines were used. A-549 are human lung adenocarcinoma cells; topotecan and other inhibitors of Top1 are used to treat this type of cancer. T98G is a human glioblastoma cell line. MCF-7 are human breast adenocarcinoma cells, characterized by its high levels of TDP1 expression.[[43]](#endnote-43) The WI-38 cell line (human fibroblast-like fetal lung cells) is an immortalized and non-cancerous used for comparison between diseased and healthy cells.

The cells were exposed to the most efficient inhibitors, with IC50 values less than 10 μM, in concentration from 0.5 μM to 100 μM. Most of compounds do not show any cytotoxic effect for concentration up to 100 μM for the cell lines: A-549 (Fig. 3A), WI-38 (Fig. 3B), T98G (Fig. 3C) and MCF-7 (Fig. 3D). Compound **7** demonstrated mild cytotoxicity in three cell lines: WI-38 cells (CC50=60 μM) (Cytotoxic Concentration at 50%, concentration killing 50% of the cells), T98G (CC50=16 μM) and MCF-7 (CC50=17 μM). Compound **10** appeared to be slightly toxic for two cell lines T98G (CC50=66 μM) and MCF-7 (CC50=80 μM)) and compound **6** only for T98G cells (CC50=16 μM) (Supplementary, Table S3). There was no obvious correlation between cytotoxicity IC50 values of the ligands.



A

B

C

D

**Fig. 3.** Dose-dependent action of the ligands on A-549 (A), WI-38 (B), T98G (C) and MCF-7 (D) cell lines. The viability was measured with the MTT assay.

**2.4. Synergistic activity with topotecan against tumor cells**

As previously explained, the ability of TDP1 to unravel the Top1-DNA stalled complex, Tdp1 activity can work against the efficacy of the Top1 poisons. Thus, blocking TDP1 has the potential to increase the efficacy of topotecan and irinotecan.[[44]](#endnote-44)

For this purpose, we evaluated the influence of the TDP1 inhibitors on the cytotoxic effect of topotecan on the A-549, WI-38 and T98G cell lines. The CC50 values of topotecan in the presence of DMSO and ligands in two concentrations were measured. The enhancement of topotecan cytotoxicity and combination indices (CI) were calculated. (Supplementary, Tables S1 and S2). CI values reflect the extent of drug-drug interaction. CI < 1 indicates synergy, CI = 1 - additivity and CI > 1 – antagonism.[[45]](#endnote-45)

The most effective inhibitors were selected that meet at least one of two requirements. The compound had to inhibit TDP1 with IC50 value less than 1 μM or SCAN1 with IC50 value less than 20 μM. Five compounds fit this criterion: **5**, **6**, **7**, **14** and **15**.

Compounds **5** and **7** potentiated the topotecan cytotoxicity 8 and 4.5 times, respectively for the A-549 cell line (Fig. 4A, red and pink plots) and showed low or no enhancement for both T98G (Supplementary, Table S1) and WI-38 cells (Fig. 5). The combination indices for these compounds (0.2 and 0.1 respectively) for A-549 cells indicate their synergy with topotecan. Compounds **5** and **7** most effectively enhance the cytotoxicity of topotecan against lung cancer cells A-549, while the ligands do not show a cytotoxic effect. Compounds **6**, **14** and **15** had low or no enhancement for all three cell lines (Fig. 4 and 5, Supplementary, Table S1).

Even though no obvious correlation between synergy with topotecan and the IC50 values was observed, based on these experiments we selected two compounds **5** and **7** that show synergy with topotecan for A-549 cell line for further research.



A B

**Fig. 4.** Dose-dependent action of topotecan in combination with 1 μM of **5**, **6**, **7** (A) and **14**, **15** (B) on A-549 cells viability.



A B

**Fig. 5.** Dose-dependent action of topotecan in combination with 1 μM of **5**, **6**, **7** (A) and **14**, **15** (B) on WI-38 cells viability.

**2.5. Molecular modelling**

The docked conformation of **5** into the binding pocket of the SCAN1 homology pocket (Fig. 6) suggests that the substituted phenyl is positioned in the hydrophobic pocket to the left, and the two coumarin moieties are positioned in the centre and the right pocket. There are four predicted hydrogen bonds, two interactions with His263 and Lys265 and two with Arg493. Additionally, hydrophobic π-π T-shaped contacts are also predicted between Tyr204 the central coumarin.

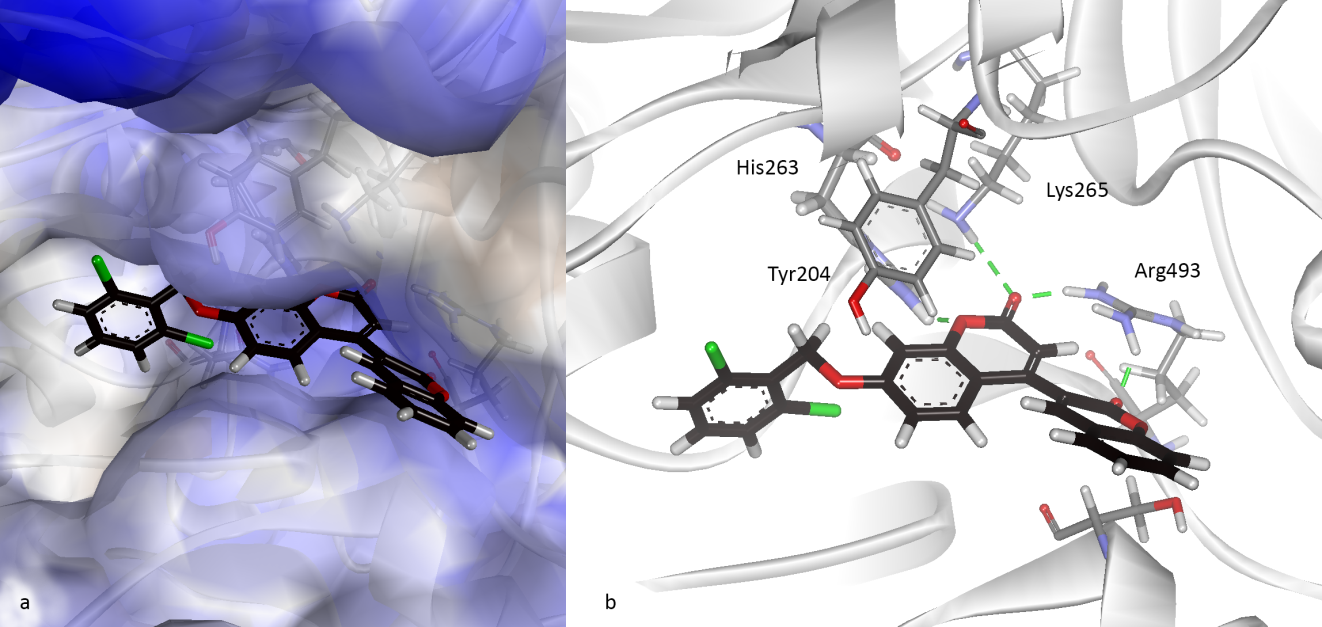


Fig. 6. a) Docked configuration of 5 to SCAN1. The surface is rendered. b) Predicted hydrogen bonding interactions of 5 with His263, Lys265 and Arg493 indicated by dashed green lines, their lengths are from 1.8 to 2.2 Å.

The closely related analogue **6** has very similar docked conformations with the single chlorine substitution having no effect on the binding. This indicates that aromatic groups are less tolerated or that the halogen species was preferred. An example of this intolerance was the inactivity of compound **8**. The naphthalene moiety is predicted to be positioned in the left of the binding region, inside the hydrophobic pocket. Previous studies with derivative **25c** indicated that a large pinene moiety was well tolerated,25 thus the idea that bulkiness of the naphthalene may be causing the inactivity seems unlikely. However, mutation to the protein may have altered the binding pocket more than predicted and modified the binding region.

Compounds **14** and **15** also share a similar structure and the docking algorithm predicted similar poses (see Fig. 7). The benzo-dioxole was positioned within the upper are of the hydrophobic pocket whilst the cyclohexene carboxamide was in the lower. The pyrido-indole was positioned within the cleft. Both compounds were predicted to have two hydrogen bonding interactions with Arg549 and Tyr204 in addition to a hydrophobic interaction with Tyr204.

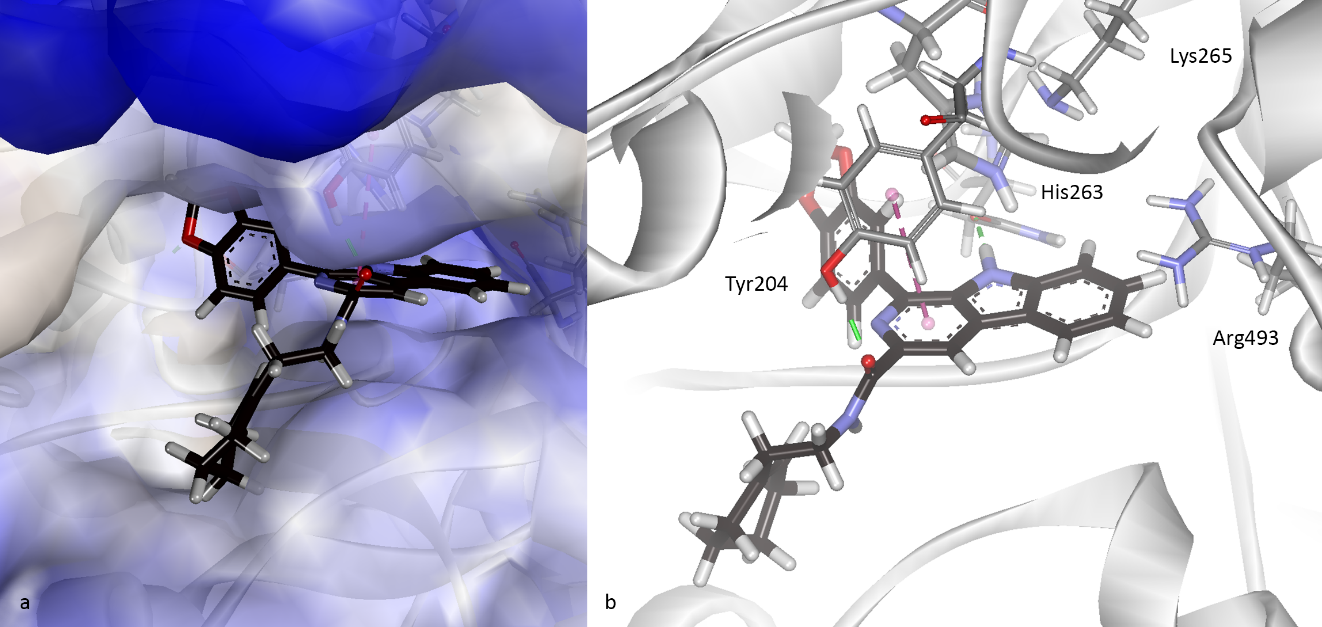


Fig 7. a) Docked configuration of 15 to SCAN1. The surface is rendered. b) Predicted hydrogen bonding interactions of 15 with Tyr204, His263 and Arg516 indicated by dashed green lines (1.8 to 2.2 Å.). Hydrophobic interactions (π-π T-shaped) with Trp590 indicated by dashed pink lines.

**2.6. Chemical Space**

The calculated molecular descriptors (MW – molecular weight, log P – octanol water partition, HD – hydrogen bond donors, HA – hydrogen bond acceptors, PSA – polar surface area and RB – rotatable bonds) for the seventeen derivatives are given in Table 2. The ligands are relatively large in size with molecular weight between 392.4 and 559.6 with most of ligands in the mid-400s. This means a majority are within the boundaries of *drug – like* chemical space (for definition of *lead-like*, *drug-like* and Known Drug Space (KDS) regions see ref.[[46]](#endnote-46)). The log P values range from 2.0 to 6.1, most of the compounds lie outside the *lead-like* region reaching into KDS.

For further analysis the Know Drug Indexes 2a and 2b (KDI2a/2b)[[47]](#endnote-47) were also derived. The KDI reflect the overall balance of the six molecular properties calculated based on the statistical distribution of KDS and derivation of an index for each descriptor. KDI2a is an additive value with a maximum of 6.0 and KDI2b the indexes are then multiplied giving 1.0 as its maximum.

The average for KDI2a for known drugs is 4.08 (±1.27) and the hits lie around the average of 5.10, the KDI2b gives a range of 0.11 to 0.69 with the average of know drugs being 0.18 (±0.20). The hits have superior values than the drugs in clinical use. However, the relatively large size in terms of MW and Log P give somewhat low values for the KDI2b index. Due to the multiplication of the molecular descriptor indexes for KDI2b it is less forgiving than KDI2a where a poor value for one descriptor can be compensated. The active dicoumarins **5** and **7** have KDI values of 5.39/5.30(2A) and 0.51/0.46(2B) with the β–carbolines **14** and **15** having even slightly better results with 5.33/5.47 (2A) and 0.47/0.54 (2B), both series having reasonable balance of their molecular descriptors indicating descent oral bioavailability.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Name | MW | HB Donor | HB Acceptor | Log P | PSA | Rotatable Bonds | KDI2a/b |
| 1 | 517.6 | 1.3 | 7.5 | 5.4 | 123.8 | 12 | 4.42/0.11 |
| 2 | 478.5 | 3.3 | 7.8 | 2.8 | 161.5 | 10 | 4.62/0.18 |
| 3 | 459.5 | 1.3 | 7.8 | 3.7 | 139.9 | 9 | 5.06/0.34 |
| 4 | 469.9 | 2.0 | 7.8 | 3.6 | 126.4 | 8 | 5.28/0.45 |
| 5 | 465.3 | 0.0 | 5.8 | 4.8 | 78.9 | 4 | 5.39/0.51 |
| 6 | 430.8 | 0.0 | 5.8 | 4.6 | 82.4 | 4 | 5.52/0.58 |
| 7 | 474.9 | 0.0 | 6.5 | 5.0 | 85.9 | 5 | 5.30/0.46 |
| 8 | 446.5 | 0.0 | 5.8 | 4.9 | 81.3 | 4 | 5.43/0.53 |
| 9 | 392.4 | 0.0 | 8.5 | 2.0 | 117.0 | 5 | 5.36/0.49 |
| 10 | 559.6 | 1.3 | 7.3 | 5.9 | 126.9 | 10 | 4.38/0.12 |
| 11 | 548.0 | 1.3 | 6.8 | 5.8 | 120.3 | 8 | 4.76/0.22 |
| 12 | 519.6 | 1.3 | 7.3 | 5.1 | 127.0 | 10 | 4.68/0.20 |
| 13 | 419.4 | 1.3 | 6.8 | 2.9 | 129.8 | 6 | 5.65/0.69 |
| 14 | 474.5 | 3.0 | 5.0 | 5.5 | 87.8 | 6 | 5.33/0.47 |
| 15 | 439.5 | 2.0 | 5.0 | 5.8 | 68.6 | 6 | 5.47/0.54 |
| 16 | 431.5 | 2.0 | 6.7 | 4.8 | 85.1 | 8 | 5.50/0.57 |
| 17 | 496.6 | 3.3 | 8.3 | 3.0 | 120.4 | 13 | 4.49/0.10 |

**Table 2**. Calculated molecular descriptors for the selected hits.

**3. Conclusions**

In this study, two chemical series, coumarin and β-carboline derivatives were identified using virtual screening against a homology model of SCAN1. Both series contain bulky substituents (*i.e.* chlorobenzene or naphthalene moieties) and exhibit both TDP1 and SCAN1 inhibition. Most of the compounds are moderately toxic or non-toxic against human cell lines. Furthermore, plausible binding modes for the active ligands are predicted as well as favorable physicochemical properties for cell permeability. These attribute makes the active ligands found in this study excellent starting points for further development as potential therapy for SCAN1 sufferers. To our best knowledge, data on inhibitors of the mutant form of TDP1 was obtained for the first time in this study.

For the inhibition mechanism, two types of inhibitors were found: those acting on the first stage of catalysis, the binding of the substrate and the formation of a complex (active against both TDP1 and SCAN1), and those acting on the second stage, the release of the enzyme (active against TDP1 only). Both types of inhibitors are suitable for creating drugs for the adjuvant cancer therapy, taking into account the possible neurotoxicity of compounds that do not affect the activity of the SCAN1 enzyme. The most promising dicoumarin derivatives **5** and **7** exhibit potent anti-TDP1 activity, low cytotoxicity, and synergism with topotecan, an established Top1 anticancer drug. *In vivo* studies will elucidate whether these compounds have a potential as an adjunctive therapy with topotecan.

**4. Experimental section**

**4.1 Molecular modeling and virtual screening**

The compounds were screened to the edited crystal structure of TDP1 (PDB ID: 1MU7, resolution 2.0 Å),28 which was obtained from the Protein Data Bank (PDB).[[48]](#endnote-48),[[49]](#endnote-49) The Scigress FJ 2.6 program[[50]](#endnote-50) was used to prepare the crystal structure for docking, *i.e.*, hydrogen atoms were added, the co-crystallised tungsten(VI)ion was removed as well as crystallographic water molecules. The amino acid His493 was converted to Arg493, the remainder of the protein backbone was locked and PM336 optimisation was run on the modified amino acid. The basic amino acids lysine and arginine were defined as protonated. Furthermore, aspartic and glutamic acids were assumed to be deprotonated. The centre of the binding pocket was defined as the position of the hydrogen atom of His263 (TDP1), which nitrogen formed a coordination bond with the tungsten ion (x = 8.312, y = 12.660, z = 35.452) with 10 Å radius. Ten docking runs were allowed for each ligand with 30% search efficiency resulting in 380 candidates. These were all re-docked using 100% search efficiency and fifty docking runs. The results were inspected visually and seventeen virtual hits selected. The GoldScore (GS),37 ChemScore (CS),38,39 Piecewise Linear Potential (ChemPLP)40 and Astex Statistical Potential (ASP)41 scoring functions were implemented to validate the predicted binding modes and relative energies of the ligands using the GOLD v5.4 software suite.

The QikProp 3.2[[51]](#endnote-51) software package was used to calculate the molecular descriptors of the compounds. The reliability of QikProp has been established for the molecular descriptors.[[52]](#endnote-52)

**4.2. General biological methods**

**4.2.1 Real-time detection of TDP1 and SCAN1 activity**

The TDP1 and SCAN1 activity measurements were carried out as previously described.21 The reaction mixtures of 200 μL contained TDP1 buffer (50 mM Tris-HCl pH8,0, 50 mM MaCl, 7 mM β-mercaptoethanol), TDP1-biosensor 5′-(5,6 FAM-AAC GTC AGG GTC TTC C-BHQ1)-3′ with a final concentration of 50 nM and inhibitors in various concentrations. All compounds were purchased in the InterBioScreen natural product collection. The reaction started when TDP1 (1.5 nM) or SCAN1 (150 nM) was added. The reaction mixtures were incubated at a temperature of 26 °C in a POLARstar OPTIMA fluorimeter, BGM LABTECH, GmbH, to measure fluorescence every 1 min (Ex485/Em520 nm). TDP1 or SCAN1 inhibition was calculated by comparing the rate of increase in fluorescence in the present of compound to that of DMSO control wells. IC50 values were determined using a concentration response curve. The data were imported into the MARS Data Analysis 2.0 program (BGM LABTECH), and the slope during the linear phase was calculated. Biosensor was synthesized in the Laboratory of Biomedical Chemistry, ICBFM SB RAS.

**4.2.2 Gel-Based Enzyme Assay**

The reaction mixtures of 200 μL contained TDP1 buffer (50 mM Tris-HCl pH8,0, 50 mM MaCl, 7 mM β-mercaptoethanol), TDP1-biosensor mentioned above with a final concentration of 50 nM and inhibitors in various concentrations. The reaction was started by adding TDP1 to a final concentration of 1.5 nM or SCAN1 to a final concentration of 150 nM. The reaction mixtures were incubated at a room temperature for 20 min. The reaction was stopped by the addition of a loading buffer.[[53]](#endnote-53)

The products were visualized using a Typhoon FLA 9500 (GE Healthcare) scanner. The quantitative calculation was carried out with the QuantityOne 4.6.7 program. The IC50 values were determined using the OriginPro 8.6.0 software.

**4.2.3 Cell culture assay**

Tumor cell lines A549 (human alveolar adenocarcinoma), T98G (human glioblastoma), MCF-7 (human breast adenocarcinoma) and WI-38 (non cancer female human embryonic lung) were plated into the wells of 96-well plate at a density ~2000 cells per well, incubated for 24h at 37 °C in IMDM medium (5% CO2) and then they were treated with the coumarin or β-carboline derivatives in four concentrations: 1, 5, 20 and 100 µM. After 72h of cell incubation, the relative amount of live cells was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (a standard colorimetric MTT-test) and the CC50 values of compounds were measured.[[54]](#endnote-54)

To determine the synergistic effect of the ligands and topotecan cells were exposed to the inhibitors in nontoxic concentrations (from 0.5 to 10 μM depending on their own CC50 values) and different concentrations of topotecan (0, 30, 100, 300, 500, 1000, 2500 nM). The CC50 values of topotecan in the presence of DMSO and compound in two concentrations were measured. The enhancement of topotecan cytotoxicity was determined as the ratio of CC50 value without TDP1 inhibitor to CC50 value with it. Combination indices (CI) were calculated according to the formula:

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where CC50,tpc and CC50,inh – the concentration of drugs needed to achieve 50% of drug effect separately; C50, tpc – the concentration of topotecan needed to achieve same effect in combination with TDP1 inhibitor; C50,inh – the concentration of inhibitor needed to achieve same effect in combination with topotecan. CI < 1 indicates synergy, CI = 1 - additivity and CI > 1 – antagonism.45 For cases when CC50 is more than 100 µM CC50,inh value assumed to be equal to 100 µM.

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**SUPPLEMENTARY**

**Synergistic activity with topotecan against tumor cells**

**Table S1.** Enhancement of topotecan cytotoxicity for T98G cells in the presence of tested compounds

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | T98G | | | | | | | |
|  | CC50 inh, μM | CC50 tpc, μM \*10-1 | CC50 tpc (when C50inh = 1 μM), μM \*10-1 | CC50 tpc (when C50inh = 10 μM), μM \*10-1 | CI (I=1 μM) | CI (I=10 μM) | Enhancement (I=1 μM) | Enhancement (I=10 μM) |
| 5 | > 100 | 2.7±0,4 | 1.9±0,3 | 1.8±0,2 | 0.7 | 0.7 | 1.4 | 1.5 |
| 6 | 16±2 | 7.2±0,7 | 4.9±0,5 | 1.4±0,1 | 0.7 | 0.5 | 1.5 | 5.1 |
| 7 | 16±2 | 13.6±1,4 | 8.6±0.9 | 7.6±0.8 | 0.7 | 1.2 | 1.6 | 1.8 |
| 14 | > 100 | 22.0±4.2 | 19.1±3.6 | 15.0±2.9 | 0.9 | 0.7 | 1.2 | 1.5 |
| 15 | > 100 | 4,3±0,4 | 9,2±0,9 | 7,9±0,8 | 2,1 | 1,9 | 0,5 | 0,5 |
| 10 | 65,8±6,7 | 1±0,1 | 1±0,1 | 1±0,1 | 1 | 1,1 | 1 | 1 |
| 1 | > 100 | 2,5±0,3 | 2,8±0,4 | 1,8±0,2 | 1,1 | 0,7 | 0,9 | 1,4 |
| 16 | > 100 | 2,5±0,6 | 1,8±0,8 | 5,2±2,0 | 0,7 | 2 | 1,4 | 0,5 |

**Table S2.** Enhancement of topotecan cytotoxicity for A-549 and WI-38 cells in the presence of tested compounds

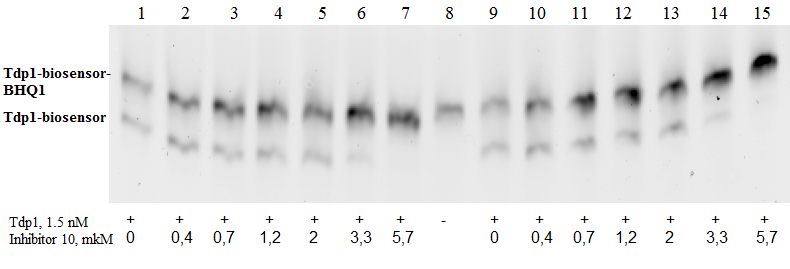
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | A549 | | | | |
| Compound | CC50 inh, μM | CC50 tpc, μM \*10-1 | CC50 tpc (when C50inh = 1 μM), μM \*10-1 | CI (I=1 μM) | Enhancement (I=1 μM) |
| 5 | > 100 | 15,0±2,3 | 3,3±0,5 | 0,2 | 4,5 |
| 6 | > 100 | 15,0±2,3 | 5,3±0,8 | 0,4 | 2,8 |
| 7 | > 100 | 15,0±2,3 | 1,9±0,3 | 0,1 | 8,0 |
| 14 | > 100 | 4,0±0,6 | 1,6±0,2 | 0,4 | 2,5 |
| 15 | > 100 | 4,0±0,6 | 7,3±1,1 | 1,8 | 0,6 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | WI-38 | | | | |
| Compound | CC50 inh, μM | CC50 tpc, nM | CC50 tpc (when C50inh = 1 μM), nM | CI (I=1 μM) | Enhancement (I=1 μM) |
| 5 | > 100 | 8,5±1,1 | 9,0±1,0 | 1 | 0,9 |
| 6 | > 100 | 8,5±1,1 | 9,5±1,0 | 1,1 | 0,9 |
| 7 | 60±6 | 8,5±1,1 | 7,0±0,8 | 0,8 | 1,2 |
| 14 | > 100 | 10,0±1,6 | 5,0±0,8 | 0,5 | 2 |
| 15 | > 100 | 10,0±1,6 | 5,0±0,8 | 0,5 | 2 |

**Table S3.** CC50 values (μM) for tested compounds against A-549, WI-38, T98G and MCF-7 cells

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | A-549 | WI-38 | T98G | MCF-7 |
| 1 | > 100 | n/d | > 100 | > 100 |
| 5 | > 100 | > 100 | > 100 | > 100 |
| 6 | > 100 | > 100 | 16±2 | > 100 |
| 7 | > 100 | 60±6 | 16±2 | 17±2 |
| 8 | 44±7 | n/d | 11±2 | 49±3 |
| 10 | > 100 | n/d | 66±7 | 80±5 |
| 14 | > 100 | > 100 | > 100 | > 100 |
| 15 | > 100 | > 100 | > 100 | > 100 |
| 16 | > 100 | n/d | > 100 | > 100 |

**Verification of IC50 values with independent gel-based electrophoresis method**



**Fig. S1.** DNA gel electrophoresis of products of the BHQ1 cleavage reaction from 3’-end of TDP1-biosensor, catalyzed by TDP1 in the presence of inhibitor **10** in different concentrations (0, 0.4, 0.7, 1.2, 2, 3.3, 5.7 µM). The reaction is conducted in two repeats: lanes 1-7 and 9-15 respectively. The control of DNA-biosensor without TDP1 and its inhibitor is in the lane 8.



**Fig. S2.** The dependence of the reaction product (DNA-biosensor without BHQ1) related to all reaction mixture expressed in percentages on the concentration of the inhibitor **10.** tau value is IC50 value of compound **10** (IC50=1.75 µM).

, when, so

**Table S4.** The results of the scoring functions for the selected compounds and their corresponding SCAN1 IC50 values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Name | GoldScore | ChemScore | ChemPLP | ASP | IC50, μM |
| 1 | 66.2 | 27.6 | 72.2 | 34.5 | > 100 |
| 2 | 71.9 | 25.1 | 66.2 | 40.3 | > 100 |
| 3 | 66.8 | 24.9 | 69.4 | 37.7 | > 100 |
| 4 | 64.6 | 28.0 | 71.9 | 32.0 | > 100 |
| 5 | 65.6 | 31.6 | 66.5 | 32.1 | 3.5±2.3 |
| 6 | 64.0 | 29.6 | 67.4 | 34.0 | 13.9±5.6 |
| 7 | 59.6 | 30.0 | 66.2 | 34.8 | 6.0±1.5 |
| 8 | 62.4 | 30.6 | 68.0 | 34.6 | > 100 |
| 9 | 63.2 | 27.0 | 70.7 | 33.7 | > 100 |
| 10 | 74.4 | 24.6 | 73.1 | 33.7 | 25.1±13.7 |
| 11 | 65.4 | 24.8 | 68.4 | 36.1 | > 100 |
| 12 | 67.0 | 30.8 | 74.4 | 32.6 | > 100 |
| 13 | 60.1 | 26.9 | 66.6 | 33.6 | > 100 |
| 14 | 65.8 | 32.0 | 69.9 | 39.2 | 17.6±6.1 |
| 15 | 62.3 | 30.7 | 65.4 | 32.6 | 14.1±1.9 |
| 16 | 58.9 | 28.2 | 67.6 | 32.8 | > 100 |
| 17 | 64.6 | 28.0 | 71.9 | 32.0 | > 100 |

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