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Discovery of Indole Derivatives as Novel and Potent Dengue Virus Inhibitors

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ABSTRACT

3-Acyl-indole derivative **1** was identified as a novel dengue virus (DENV) inhibitor from a DENV serotype 2 (DENV-2) phenotypic antiviral screen. Extensive SAR studies led to the discovery of new derivatives with improved DENV-2 potency as well as activity in nanomolar to micromolar range against the other DENV serotypes. In addition to the potency, physicochemical properties and metabolic stability in rat and human microsomes were improved during the optimization process.

Chiral separation of the racemic mixtures showed a clear preference for one of the two enantiomers. Furthermore, rat pharmacokinetics of 2 compounds will be discussed in more detail demonstrating the potential of this new series of pan-serotype-DENV inhibitors.

INTRODUCTION

Infections with dengue virus (DENV) can result in dengue fever that can further develop into severe dengue such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS); a potentially lethal condition if left untreated. Annually, an estimated 390 million infections with DENV occur in tropical and sub-tropical areas worldwide, of which roughly a quarter (~96 million) develop symptoms which is typically a painful, debilitating disease with high fever, headache, retro-orbital pain, general malaise, rash, nausea, vomiting and joint/bone/muscle pain ('breakbone' fever)[1]. A proportion of the cases (~500 000) progresses into severe dengue for which treatment is limited to symptomatic alleviation and supportive care and an estimated 22 000 people succumb to DENV infections each year. Because of the increase in the geographical distribution of the DENV vector (the *Aedes* mosquito), the urbanization and globalization, the incidence and prevalence of dengue cases have exploded in the last decades and are expected to rise even more[2-4]. Currently, ~2.5-3.0 billion people living in (sub)tropical regions are at risk to become infected with DENV[4]. The virus forms a tremendous health and socio-economic burden in the affected regions and extra measures to control the dramatic spread of the virus are urgently needed. Recently a DENV vaccine (CYD-TDV from Sanofi-Pasteur) has been licensed in a number of countries but it has rather limited efficacy and some safety issues[5-6]. Because of that, CYD-TDV is contra-indicated for use in individuals with a seronegative status as well as in young children (<5 years), which are also thought to be primarily seronegative for dengue. Via a

process called antibody-dependent enhancement (ADE), the vaccine is believed to sensitize naive recipients, resulting in an enhanced DENV infection when exposed to DENV for the first time[7]. Vaccination could then lead to an opposite effect than for which it was originally designed, i.e. more cases of hospitalized dengue (DHS and DSS) instead of fewer cases. Hence, a dengue vaccine or dengue antiviral should be equally effective against all four DENV serotypes[6]. Potent inhibitors of the virus could serve multiple purposes such as prophylactic treatment for travelers to and people living in dengue-endemic regions, household prophylaxis when there is an index case and treatment of patients with an ongoing DENV infection. Despite efforts performed by different research groups[8-13], there are still no antiviral drugs available for the treatment and/or prophylaxis of DENV infections. The clinical development of the majority of these dengue molecules was halted because of limited or low efficacy against some of the four dengue serotypes[12], limited solubility[13] or other pharmacokinetic and/or safety issues in animals[10]. In an effort to identify novel small molecules acting as dengue virus inhibitors, a medium-throughput phenotypic antiviral screening using a cytopathic effect (CPE) reduction assay against DENV-2 ('Rega' lab strain) was performed with the CD3 (Centre for Drug Design and Discovery, KULeuven) compound library. This effort led to the identification of 2-((3,4-dimethoxyphenyl)amino)-1-(1*H*-indol-3-yl)-2-phenylethan-1-one (Compound (**1**), Figure 1) as a novel dengue virus inhibitor. Using a virus yield reduction assay (RT-qPCR readout) as confirmatory assay, compound **1** demonstrated potent and genuine *in vitro* antiviral activity against DENV-2 (EC_{50} = 0.078 μM) and was shown to significantly reduce viral RNA replication ($3.8\log_{10}$) at non-toxic concentrations (data not shown), indicating that the antiviral activity is selective and is thus not associated with an adverse/pleiotropic effect on the host cell. In terms of mechanism of action, selection of in *vitro drug*-resistant variants led to the

identification of multiple mutations in the viral NS4B sequence that are required to be present together to result in phenotypic resistance. A manuscript describing the mechanism of action studies for this class of compounds is in preparation and will be published elsewhere. As the potential molecular target (NS4B) of compound **1** does not exert any enzymatic activity and the structure of this membrane associated protein has not been solved, the optimization of the antiviral activity was performed by systematic modifications of the constituent parts of compound **1** (Figure 2) using the DENV-2 virus yield reduction assay with RT-qPCR readout for activity determination. In this paper, we describe how SAR investigations led to compounds with nanomolar to micromolar activity against the 4 DENV serotypes. In addition, we report on further optimization of the series resulting in compounds with a good pharmacokinetic profile and improved oral bioavailability in rat.

CHEMISTRY

Two synthetic routes were developed to prepare the desired ketoindole derivatives **1** and **10-56**, as depicted in Scheme 1. The first synthetic route was based on the cross-coupling of aldehydes with imines catalyzed by an *N*-heterocyclic carbene as described by Li *et al.* [14]. Starting from imines **4** (generally synthesized by heating of an aldehyde **2** and an amine **3**) and BOC-protected indole-3-carboxaldehydes **5**, this one-pot procedure allowed us to generate a diverse library of final compounds bearing modifications on R^1, R^2, and R^3. During the establishment of the condensation reaction between the imines **4** and the aldehydes **5**, it appeared that the protection of the indole NH was required. The BOC group turned to be the most suitable protecting group as it could be removed easily in a few minutes at 160 °C under microwave irradiation (MW) without obvious decomposition. It also seemed to activate the aldehyde in 3-

position, resulting in better yields than other protection groups such as benzyl. However, this Umpolung reaction failed to give the desired products when using alkyl- or cycloalkyl-aldehydes **2** or some electron-rich anilines **3** such as dimethoxyanilines. Therefore, a new three-step synthesis approach was developed to access other compounds of interest. Friedel-Crafts reaction with acyl chloride **7** in the presence of Et_2AlCl[15] as Lewis acid proceeded regioselectively in 3-position of unprotected indoles **6** to give 3-acylindole derivatives **8** in good yields. Bromination in α-position of the carbonyl group with phenyltrimethylammonium tribromide[16] followed by bromine displacement with amines **3** provided the desired final compounds. Scheme 2 illustrates the synthesis of compounds **61-62**, **76-79** and **91-92**. Compounds **61** and **62** with an hydroxymethyl group were prepared by acylation of **59** and **60** with α-chlorophenylacetyl chloride in pyridine[17] followed by chlorine displacement with 3,5-di-methoxyaniline under microwave irradiation. It is worth mentioning that the acylation conditions used in Scheme 1 led to a complex mixture of compounds. The hydroxyethyl-indoles **67** and **68** were prepared from indole carboxaldehydes **63** and **64** by a Wittig reaction to get the corresponding vinyl indoles followed by hydroboration. Further conversions of the hydroxyl group in dimethylamino or methyl sulfone were performed on the 3-acylindole **73**. The hydroxypropyl chain on the indole was constructed in 3 steps comprising a Horner-Wadsworth-Emmons reaction of indole carboxaldehydes **63** and **80** to get the methyl acrylate derivatives **81** and **82**, followed by the reduction of the double bond by hydrogenation and ester reduction with $LiBH_4$. Scheme 3 outlines the synthesis of the anilines substituted with alkoxy chains containing a hydroxy or an amino group. Selective mono-demethylation of 3,5-dimethoxyaniline **93** with dodecanethiol and NaOH in NMP[18] provided 3-amino-5-methoxyphenol **94**. *O*-Alkylation with bromoalkyls or alkylsulfonates afforded the desired anilines.

RESULTS AND DISCUSSION

Early structure-activity relationship (SAR) investigation. The SAR was explored with the conceptual division of hit **1** into three distinct structural subunits: the 3,4-di-methoxyaniline, the phenyl head part and the indole core (Figure 2). The DENV-2 antiviral activity of all newly synthesized compounds was determined together with their cellular cytotoxicity.

First, we investigated aniline modifications (Table 1) by varying substituents or substitution patterns on the aromatic ring (**10**-**21**). Aniline replacement with heteroaromatic or cycloalkyl amines was also investigated (**22**-**28**). A survey of the dimethoxy anilines revealed that the substitution pattern was essential for the activity. Indeed, the 3,5-dimethoxyaniline analogue **12** showed a ten-fold increase of antiviral activity compared to **1** and was amongst the most potent compounds within the series (EC_{50} = 0.007 μM), while the 2,5- and 2,3-dimethoxyaniline derivatives only showed submicromolar antiviral activity (**10**-**11**). In addition to the beneficial impact on potency, the 3,5-dimethoxyaniline moiety offers the advantage of lacking a methoxy group in para position of the amine, which could pose a safety concern if metabolized in iminoquinone.[19-20] Combination of a 3-methoxy and another substituent in 5-position on the aniline, such as an ethoxy **13** or a fluorine **14**, was well tolerated whereas the 3,5-difluoroaniline derivative **15** showed a 85-fold decrease in potency compared to **14**. This result clearly indicates that at least one methoxy group in *meta* position of the aniline is required to get DENV-2 double digit nanomolar potency. Moreover, the crucial role of a methoxy in *meta* position of the aniline was confirmed with mono-substituted aniline derivatives. Indeed, the substitution of the *meta* methoxy (compound **16**) by a trifluoromethoxy, cyano, fluoro or methyl sulfone (**17**-**20)** was associated with a decrease of antiviral activity (12- to 90-fold, respectively). Similarly, the key

role of one *meta* methoxy substituent was demonstrated in the pyridyl series (compounds **22** and **23**). Furthermore, other heteroarylamines, even those bearing a methoxy group (**24**-**27**), cycloalkylamines such as **28** and alkylamines (data not shown) were associated with a reduced potency.

We next investigated modifications around the phenyl head part (Table 2). To evaluate the effect of substituents on the phenyl ring, we synthesized compounds **29**-**35**. Introduction of one methyl **29**, fluorine **32** or cyano **34** in *para*-position led to a 2- to 3-fold improvement in activity compared to **16**. The nature of the substituents on this *para* position proved to be important for potency and could lead to a strong reduction in activity as demonstrated by the methyl sulfone derivative **35**. Comparing the activities of the *ortho*-, *meta*- or *para*-methyl derivatives **29**-**31**, *para*-substitution seemed to be preferred, *ortho*-substitution was well tolerated whereas *meta*-substitution led to a 12-fold decrease in activity. We also investigated the effect of six-membered (**36**; **38**-**41**), five-membered (**42**-**44**) and bicyclic (**45**-**47**) heteroaromatic rings. 3- or 4-Pyridyl derivatives **36** and **39** were the 6-membered heteroaromatic rings best tolerated and substitution of the 3-pyridyl with a methyl (**38**) further increased the activity. Bioisosteric replacement[21-22] of the phenyl ring by a thienyl **42** or a furyl **43** led to compounds with similar potency whereas other five-membered heteroaryl rings such as oxadiazole **44** were less active. An interesting improvement in potency could be achieved with some bicycles. Benzoxazole and imidazopyridine derivatives **45** and **46** were significantly more potent than the phenyl derivative **16** with antiviral activities in the low nanomolar range. Combining the modifications of the phenyl head part with the aniline associated with the best potency (3,5-dimethoxyaniline) led to at least a 5-fold improvement in activity as observed with compounds **33** and **37.**

A variety of analogues (**48**-**56**) were synthesized to evaluate the effect of substitution in 4-, 5-, 6- and 7-position of the indole core (Table 3). Introduction of halogens, methoxy or methyl groups was well tolerated in all positions. Direct matched pair analysis of 4- and 6-chloro and fluoro substituted indoles (**48**, **49**, **52** and **53**) showed a clear preference for chlorine over fluorine. The most potent compound with a 6-chloroindole **53** presented an EC_{50} value of 0.013 μM against DENV-2 with a high SI (1000). Combining a substituted indole with the 3,5-dimethoxyaniline yielded a significantly more potent compound with a 5-fold improvement in activity (**50** and **51**).

Having identified key modifications on the three structural subunits which resulted in about 20-fold improved potency (**1** vs. **12**), we turned our attention to the chiral center and its influence on the DENV-2 antiviral activity. Considering the difficulties to synthesize the compounds in an enantioselective manner, we decided to use chiral HPLC to separate the enantiomers from a racemic mixture. To this end, **12** was selected as test compound and was screened on several chiral columns. A good separation could be achieved (see Experimental Part) leading to both enantiomers **12a** and **12b** which were evaluated in our DENV-2 assay together with **12** (Table 4). Albeit having a similar cytotoxicity, the two enantiomers showed a marked difference in potency with the (+)-enantiomer **12a** being 300-fold more potent than the (-)-enantiomer **12b**. Until now, we were not able to determine the absolute configuration of the most active enantiomer.

Broad-spectrum evaluation. One challenge in the development of small-molecule dengue inhibitors is that compounds should be potent against the four dengue serotypes[23]. The antiviral effect of the hit **1** and a selection of potent compounds against the DENV-2 'Rega' lab strain (**12a**, **37** and **51**) was evaluated against clinical isolates or lab-adapted strains representing the

four dengue serotypes (Table 5). While **1** showed potent activity against DENV-2, it was only weakly active against the three other tested dengue serotypes with EC_{50} values in the micromolar range. Interestingly, compounds showing improved DENV-2 activities also presented an improved profile against DENV-1 and DENV-3. However, the DENV-4 activity remained in the micromolar range despite the noticeable improvements achieved against the other 3 serotypes.

***In vitro* ADMET and PK.** Compounds were selected based on their antiviral potency and chemical diversity for a detailed evaluation of their *in vitro* ADMET properties (Table 6). Compound **12** displayed low solubility and moderate permeability. Metabolic stability was different across the species with high stability in human liver microsomes (HLM) and moderate stability in rat liver microsomes (RLM). **12** did not show significant hERG inhibition. However, there was a consistent single digit micromolar inhibition of CYP1A2, 2C19 and 2C9 (IC_{50} > 10μM for 2D6 and 3A4). The two enantiomers **12a** and **12b** showed a similar profile regarding solubility, permeability, plasma protein binding, CYP inhibition and metabolic stability in HLM. However, the most potent enantiomer **12a** presented the best metabolic stability in RLM (37% turnover *vs* 91% for **12b** and 63% for the racemate **12**). A significant solubility improvement was observed for compound **23** due to the introduction of the 3-amino-5-methoxypyridine instead of the 3,5-dimethoxyaniline. Unfortunately, this gain in solubility was associated with a more pronounced hERG inhibition (IC_{50} = 3.5 μM) as well as a strong CYP3A4 inhibition (IC_{50} = 0.05 μM). Compound **45** with a benzoxazole instead of the phenyl head part proved to be very potent against DENV-2 (EC_{50} = 0.011 μM) but its further development was hampered because of its unfavorable ADMET profile (poor solubility and poor metabolic stability in RLM).

Since most of the compounds contain an aniline moiety, we decided to evaluate the potential for mutagenicity.[24-26] In that respect, several active compounds bearing more or less electron-rich moieties (**1**, **12, 16, 23** and **51**) were evaluated in an Ames test in presence or absence of human liver S9 fraction and all results proved to be negative (data not shown).

Overall **12a** presented the most interesting profile (antiviral activity and ADMET) and was therefore selected for rat pharmacokinetics (Table 7). Despite a moderate turnover in RLM (37%), the clearance after iv administration turned out to be high and we therefore decided to check the stability of the compound in rat hepatocytes to see whether phase II metabolism could possibly explain the discrepancy between our *in vitro* and *in vivo* data. Although **12a** was devoid of polar groups, it appeared to be quickly turned over in rat hepatocytes (98% of compound metabolized after 1 h), which could explain the rapid clearance observed upon iv dosing. Oral dosing led to a very low Cmax and exposure respective to the given dose, which could probably be attributed to a poor absorption. Consequently, a low oral bioavailability (7%) was calculated.

Improvement of oral bioavailability. The limited oral bioavailability could be improved by increasing the absorption or by reducing the first-pass effect or by a combination of both. To overcome absorption issue in the series, we decided to introduce polar chains on the scaffold since we suspected that the low absorption could be limited by the low aqueous solubility.

Since the SAR revealed that substituents on the indole core were well tolerated, we first focused on introducing alkyl chains bearing hydroxy, amino or sulfonyl groups. Table 8 summarizes the biological activities of all compounds bearing polar chains on the indole as well as solubility and HLM data for the most potent compounds. Introduction of hydroxyalkyl chains in 4-position led to decreased potency. The 5-position seemed to be the most suitable position for

the polar chains as the 3 compounds **62**, **76** and **92** showed interesting activity with EC_{50} values ranging between 0.096 μM and 0.181 μM. The most active compound **92** with a hydroxypropyl chain displayed an improved solubility compared to **12** but also a lower metabolic stability in HLM. Compound **77** with a hydroxyethyl chain in 7-position presented a promising activity below 0.1 μM but unfortunately also exhibited limited solubility. Derivatives **78** and **79** with a dimethylamino or methylsulfonyl group on the chain were less active. Being unable to maintain an acceptable level of potency and a better aqueous solubility with polar side chains on the indole, we decided to look at other parts of the compounds.

As compound **13** with a 3-ethoxy-5-methoxy-aniline and compound **12** with a 3,5-di-methoxyaniline showed similar activity (Table 1), we thought that combining one methoxy group and an elongated polar chain on the aniline could lead to potent compounds with better aqueous solubility. Except for compound **102** bearing a dimethylamino group, all the synthesized compounds with a polar chain on the aniline showed DENV-2 activities below 0.1 μM (Table 9). Amongst the hydroxylated chains, the most promising group seemed to be the glycol (**100**) with a positive impact on solubility and metabolic stability. Compared to **12**, compound **100** displayed a 6-fold better solubility, a similar high stability in HLM and an improved stability in RLM while maintaining a decent antiviral potency. Activity and metabolic stability could be further improved when combining the aniline with the glycol chain and one of the best head part identified in early SAR (*para*-fluoro-phenyl) in compound **101** but with a slight decrease in solubility. This clearly indicated that there is still room for optimization to get compounds with better antiviral and ADME properties. Following the good profile of **100**, its enantiomers were separated by chiral SFC. The most active enantiomer **100a** showed potent antiviral activity with a DENV-2 EC_{50} value of 0.012 μM and a better metabolic stability in RLM than the racemate

(21% turnover). The metabolic stability of **100a** was also evaluated in rat hepatocytes and was found to be moderate with 60% metabolized after 60 minutes. Based on its improved ADME profile compared to **12a** (see table 6), **100a** was selected for rat PK (Table 7). As anticipated, its improved solubility and metabolic stability translated into an interesting rat PK profile. The low turnover in RLM and moderate stability in rat hepatocytes correlated with a moderate iv clearance. The oral dose gave a 9-fold increase in Cmax compared to **12a** and a 19-fold increase in AUC with an oral bioavailability of 64%. These results clearly demonstrate the importance of identifying compounds with an acceptable aqueous solubility and high metabolic stability to reach acceptable levels of exposure *in vivo*. The PK profile of **100a** shows the potential for these novel dengue inhibitors in view of further optimization and *in vivo* studies.

CONCLUSION

In summary, we discovered and optimized a new class of indole derivatives as potent and selective inhibitors of dengue virus replication. Throughout a systematic SAR exploration, potency was improved leading to compounds with *in vitro* activity in the nanomolar to micromolar range against each of the four dengue serotypes. Chiral separation of two racemic mixtures by HPLC or SFC revealed a marked difference in potency between the two enantiomers. Despite a first rather unfavorable iv and oral rat PK profile further optimization focusing on improving solubility and metabolic stability led to the identification of **100a** which has an interesting PK profile in rats. Based on these favorable results, further optimization aiming at the improvement of the pan-serotypic coverage as well as the ADMET and PK properties has been initiated. The results (SAR, PK and *in vivo* studies) will be reported in further studies.

EXPERIMENTAL PART

Chemistry. All reagents and solvents were purchased from commercial sources and used without further purification. Flash chromatography purifications were performed on Biotage prepacked silica gel columns using Biotage Isolera or SP4 instruments. TLC was carried out with Macherey-Nagel Alugram Sil G/UV_{254} plates. TLC plates were revealed with UV light, $KMnO_4$, *p*-anisaldehyde or ninhydrine solutions. ^{1}H NMR spectra were recorded on a 300 MHz Avance, a 400 MHz Ascend or a 500 MHz Bruker spectrometer. Proton chemical shifts are reported in parts per million (δ) using TMS as a standard. Electrospray mass (ESI) measurements were obtained on a Brucker Esquire 6000 mass spectrometer. The purity of all compounds screened in biological assays was > 95 %. Purity was determined by LC-MS recorded on a system consisting of a Dionex Ultimate 3000 HPLC equipped with a PDA detector and a Brucker Esquire 6000 mass spectrometer, using a C-18 column (SunFire C18, 3.5 µm, 3.0 x 100 mm or XBridge C18, 3.5 µm, 3.0 x 100 mm). The enantiomer separations mentioned in this experimental part were carried out by chiral HPLC or chiral SFC. Optical rotations were measured on a Perkin-Elmer 341 polarimeter with a sodium lamp and reported as follows: $[\alpha]_D^T$ (λ, c g/100ml, solvent, TºC).

General procedure for Friedel-Crafts reaction. Exemplified for 1-(1*H*-indol-3-yl)-2-phenylethanone (8; R^1 = Ph, R^3 = H). To a solution of 1*H*-indole (2.5 g; 21.3 mmol) in CH_2Cl_2 (80 mL) cooled at 0 °C was added dropwise a 1M Et_2AlCl solution in hexane (32 mL; 32.0 mmol). After 30 min at 0 °C, a solution of phenylacetyl chloride (4.3 mL; 32.3 mmol) in CH_2Cl_2 (80 mL) was added. The reaction mixture was stirred at 0 °C for 3 h. 1M Rochelle salt solution was added slowly at 0 °C and the reaction mixture was stirred at rt for 2 h. The phases were

separated. The organic phase was washed with sat. $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was triturated with EtOAc to give 3.2 g (65%) of the title compound as a white powder. ESI/APCI(+): 236 (M+H). ESI/APCI(-): 234 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.01 (br s, 1H), 8.53 (s, 1H), 8.16 (d, J = 7.2 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.25 - 7.41 (m, 4H), 7.11 - 7.25 (m, 3H), 4.16 (s, 2H).

General procedure for bromination reaction. Exemplified for 2-bromo-1-(1*H*-indol-3-yl)-2-phenylethanone (9; R^1 = Ph, R^3 = H). To a solution of **8** (3.2 g; 13.8 mmol) in THF (140 mL) cooled at 0 °C was added dropwise a solution of phenyltrimethylammonium tribromide (5.7 g; 15.2 mmol) in THF (70 mL). The reaction mixture was stirred at 0 °C for 1 h and at rt overnight. The solids were filtered and washed with EtOAc. The filtrate was concentrated under reduced pressure. The residue was triturated with EtOAc to give 3.7 g (85%) of the title compound as a white solid. ESI/APCI(+): 314, 316 (M+H). ESI/APCI(-): 312, 314 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.21 (br s, 1H), 8.67 (d, J = 3.0 Hz, 1H), 8.19 (d, J = 6.4 Hz, 1H), 7.68 (d, J = 6.4 Hz, 1H), 7.14 - 7.43 (m, 7H), 6.84 (s, 1H).

General procedure for bromine displacement. Exemplified for 1-(1*H*-indol-3-yl)-2-((3-methoxyphenyl)amino)-2-phenylethanone (16). A mixture of **9** (0.032 g; 0.102 mmol) and *m*-anisidine (0.063 mL; 0.563 mmol) in acetonitrile (0.5 mL) was irradiated in a microwave oven at 100 °C for 15 min. The reaction mixture was concentrated. The residue was partitioned between EtOAc and 1N HCl. The phases were separated. The organic phase was washed with sat. $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (20% to 60%) in heptane furnished 0.030 g (83%) of the title product as a white powder. ESI/APCI(+): 357

(M+H). ESI/APCI(-): 355 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.14 (br s, 1H), 8.89 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.09 - 7.25 (m, 3H), 6.91 (t, *J* = 8.1 Hz, 1H), 6.38 - 6.50 (m, 2H), 6.35 (d, *J* = 7.9 Hz, 1H), 6.10 (d, *J* = 7.9 Hz, 2H), 3.62 (s, 3H).

General procedure for Umpolung reaction. Exemplified for 1-(1*H*-indol-3-yl)-2-((3-methoxyphenyl)amino)-2-(pyrazolo[1,5-*a*]pyridin-2-yl)ethan-1-one (46). A solution of 3-methoxy-*N*-(pyrazolo[1,5-*a*]pyridin-2-ylmethylene)aniline in ethanol was prepared by heating pyrazolo[1,5-*a*]pyridine-2-carbaldehyde (0.087 g; 0.595 mmol) and 3-methoxyaniline (0.067 mL; 0.599 mmol) in EtOH (1 mL) at 60 °C for 6 h.

To a solution of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium chloride (0.082 g; 0.304 mmol) in EtOH (0.7 mL) heated at 70 °C was added NEt_3 (0.060 mL; 0.430 mmol). After 5 min stirring, *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (0.147 g; 0.600 mmol) and the solution of imine prepared above (0.599 mmol) were added. The reaction mixture was stirred at 70 °C overnight, after which the reaction mixture was irradiated in a microwave oven at 160 °C for 4 min. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (10% to 60%) in heptane followed by precipitation from CH_2Cl_2 furnished 0.055 g (23%) of the title compound as a yellow solid. ESI/APCI(+): 397 (M+H). ESI/APCI(-): 395 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.07 (br s, 1H), 8.77 (s, 1H), 8.59 (d, *J* = 7.1 Hz, 1H), 8.18 (d, *J* = 7.1 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.47 (d, *J* = 7.1 Hz, 1H), 7.09 - 7.26 (m, 3H), 6.94 (t, *J* = 8.3 Hz, 1H), 6.81 (t, *J* = 7.1 Hz, 1H), 6.65 (s, 1H), 6.41 - 6.50 (m, 2H), 6.21 - 6.34 (m, 2H), 6.14 (d, *J* = 7.6 Hz, 1H), 3.64 (s, 3H).

Enantiomers separation of 12. Racemic **12** was subjected to chiral HPLC purification (ChiralPak IC column; eluent: *n*-heptane/dichloromethane/ethanol/diethylamine: 50/50/1/0.1) to provide **12a** (retention time = 8.1 min) and **12b** (retention time = 17.9 min). **12a:** ESI/APCI(+): 387 (M+H). ESI/APCI(-): 385 (M-H). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.14 (br s, 1H), 8.89 (s, 1H), 8.15 (d, *J* = 7.3 Hz, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.13 - 7.23 (m, 3H), 6.35 (d, *J* = 8.2 Hz, 1H), 6.07 (d, *J* = 8.2 Hz, 1H), 6.04 (d, *J* = 1.9 Hz, 2H), 5.70 (t, *J* = 2.0 Hz, 1H), 3.61 (s, 6H). ee > 99%. $[\alpha]_D^{20}$ = +44.2° (589 nm, c 0.251 w/v %, DMF, 20°C). **12b:** ESI/APCI(+): 387 (M+H). ESI/APCI(-): 385 (M-H). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.14 (br s, 1H), 8.89 (s, 1H), 8.15 (dd, *J* = 7.3, 1.1 Hz, 1H), 7.63 (d, *J* = 7.3 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 2H), 7.12 - 7.23 (m, 3H), 6.35 (d, *J* = 8.2 Hz, 1H), 6.07 (d, *J* = 7.9 Hz, 1H), 6.04 (d, *J* = 2.2 Hz, 2H), 5.71 (t, *J* = 2.0 Hz, 1H), 3.61 (s, 6H). ee > 99%. $[\alpha]_D^{20}$ = -44.1° (589 nm, c 0.213, DMF, 20°C).

General procedure for acylation with α-chlorophenylacetyl chloride followed by chlorine displacement. Exemplified for 2-((3,5-dimethoxyphenyl)amino)-1-(4-(hydroxymethyl)-1*H*-indol-3-yl)-2-phenylethanone (61). To a solution of **59** (1.0 g; 3.8 mmol) and pyridine (0.300 mL; 3.7 mmol) in toluene (10 mL) heated at 55 °C was added dropwise α-chlorophenylacetyl chloride (0.552 mL; 3.8 mmol). The reaction mixture was heated at 55 °C for 1 h. After cooling to rt, water and MeOH were added. The reaction mixture was stirred at rt for 1 h. The reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (15% to 70%) in heptane furnished 0.648 g (41%) of 1-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-1*H*-indol-3-yl)-2-chloro-2-phenylethanone as a brown oil and 0.317 g (28%) of 2-chloro-1-(4-(hydroxymethyl)-1H-indol-3-yl)-2-phenylethanone.

2-Chloro-1-(4-(hydroxymethyl)-1H-indol-3-yl)-2-phenylethanone. ESI/APCI(-): 298 (M-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.26 (br s, 1H), 8.67 (d, J = 3.4 Hz, 1H), 7.58 (d, J = 7.0 Hz, 2H), 7.26 - 7.41 (m, 5H), 7.21 (t, J = 7.7 Hz, 1H), 6.88 (s, 1H), 5.08 (dd, J = 14.7, 5.8 Hz, 1H), 4.97 (t, J = 5.7 Hz, 1H), 4.79 (dd, J = 14.7, 5.8 Hz, 1H).

A mixture of 2-chloro-1-(4-(hydroxymethyl)-1*H*-indol-3-yl)-2-phenylethanone (0.150 g; 0.500 mmol) and 3,5-dimethoxyaniline (0.767 g; 5.0 mmol) in CH_3CN (3 mL) was irradiated in a microwave oven at 130 °C for 15 min. The reaction mixture was concentrated. The residue was partitioned between EtOAc and 1N HCl. The phases were separated. The organic phase was washed with sat. $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (2% to 40%) in heptane followed by precipitation from CH_2Cl_2 furnished 0.028 g (14%) of the title product as a white powder. ESI/APCI(+): 417 (M+H). ESI/APCI(-): 415 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.24 (br s, 1H), 8.96 (s, 1H), 7.60 (d, J = 7.5 Hz, 2H), 7.25 - 7.43 (m, 4H), 7.13 - 7.25 (m, 2H), 6.36 (d, J = 7.9 Hz, 1H), 6.17 (d, J = 7.9 Hz, 1H), 6.08 (s, 2H), 4.91 - 5.17 (m, 2H), 4.65 (dd, J = 14.1, 5.5 Hz, 1H), 3.64 (s, 6H).

2-(1*H*-Indol-7-yl)ethanol (68). To a suspension of **64** (1.00 g; 6.9 mmol) and methyltriphenylphosphonium bromide (2.96 g; 8.3 mmol) in THF (40 mL) cooled at 0 °C was added dropwise a solution of *t*BuOK (1.17 g; 10.4 mmol) in THF (10 mL). The reaction mixture was stirred at rt overnight. The reaction mixture was poured into a mixture ice/water and was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (10% to 50%) in heptane furnished 0.914 g of **66** as a pink oil. ESI/APCI(+): 144 (M+H).

To a solution of **66** (0.914 g; 6.4 mmol) in THF (37 mL) cooled at 0 °C was added dropwise a 1M borane-tetrahydrofuran complex solution in THF (6.4 mL ; 6.4 mmol). The reaction mixture was stirred at rt for 2.5 h. 10% NaOH (3.1 mL; 7.8 mmol) and 30% H_2O_2 (0.869 mL; 9.8 mmol) were added. The reaction mixture was stirred at rt for 4 h. Sat. NH_4Cl was added. After 5 min stirring at rt, the reaction mixture was extracted with EtOAc. The organic phase was washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (20% to 70%) in heptane furnished 0.490 g (44% over two steps) of the title product as a brown oil. ESI/APCI(+): 162 (M+H). ESI/APCI(-): 160 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.00 (br s, 1H), 7.37 (t, J = 4.3 Hz, 1H), 7.29 (t, J = 2.8 Hz, 1H), 6.90 (d, J = 4.5 Hz, 2H), 6.35 - 6.47 (m, 1H), 4.67 (t, J = 4.9 Hz, 1H), 3.71 (q, 2H), 3.00 (t, J = 7.2 Hz, 2H).

7-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-1*H*-indole (70). To a solution of **68** (0.200 g; 1.2 mmol) in THF (4.6 mL) were added DBU (0.280 mL; 1.9 mmol) and TBDMSCl (0.412 g; 2.7 mmol). After 4 h at rt, the reaction mixture was diluted with EtOAc and washed with water. The phases were separated. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (20% to 80%) in heptane furnished 0.288 g (84%) of the title product as a brown oil. ESI/APCI(+): 276 (M+H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.02 (br s, 1H), 7.37 (t, J = 4.5 Hz, 1H), 7.29 (t, J = 2.8 Hz, 1H), 6.90 (d, J = 4.5 Hz, 2H), 6.34 – 6.46 (m, 1H), 3.87 (t, J = 6.7 Hz, 2H), 3.04 (t, J = 6.8 Hz, 2H), 0.91 (s, 9H), -0.10 (s, 6H).

1-(7-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-1*H*-indol-3-yl)-2-phenylethanone (72). This compound was synthesized in a similar manner as described for **8**. ESI/APCI(+): 394 (M+H). ESI/APCI(-): 392 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.96 (br s, 1H), 8.64 (s, 1H),

8.01 (d, *J* = 7.2 Hz, 1H), 7.24 -7.42 (m, 4H), 7.21 (d, *J* = 7.2 Hz, 1H), 6.96 - 7.14 (m, 2H), 4.15 (s, 2H), 3.85 (t, *J* = 6.6 Hz, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 0.77 (s, 9H), -0.15 (s, 6H).

2-((3,5-Dimethoxyphenyl)amino)-1-(7-(2-hydroxyethyl)-1*H*-indol-3-yl)-2-phenylethanone (77). This compound was synthesized in two steps following general procedures for bromination and bromine displacement. ESI/APCI(+): 431 (M+H). ESI/APCI(-): 429 (M-H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.04 (br s, 1H), 8.86 (s, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.28 (t, *J* = 7.1 Hz, 2H), 7.13 - 7.22 (m, 1H), 6.99 - 7.13 (m, 2H), 6.33 (d, *J* = 7.9 Hz, 1H), 6.10 (d, *J* = 7.9 Hz, 1H), 6.05 (d, *J* = 1.9 Hz, 2H), 5.71 (s, 1H), 4.69 (br s, 1H), 3.69 (t, *J* = 6.8 Hz, 2H), 3.61 (s, 6H), 3.00 (t, *J* = 6.8 Hz, 2H).

3-Amino-5-methoxyphenol (94). To a solution of **93** (2.00 g; 13.1 mmol) in NMP (10 mL) were added dodecanethiol (5.28 g; 26.1 mmol) and NaOH (1.15 g; 28.2 mmol). The reaction mixture was irradiated at 160 °C for 1 h in a microwave oven. The reaction mixture was allowed to cool to rt. The reaction mixture was diluted with sat. NaH_2PO_4 and brine and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a biphasic system. CH_3CN was added and both phases were separated. The CH_3CN layer was concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of EtOAc (25% to 75%) in heptane furnished 1.43 g (79%) of the title compound as a beige solid. ESI/APCI (+): 140 (M+H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.88 (s, 1H), 5.61 (br s, 2H), 5.53 (s, 1H), 4.91 (br s, 2H), 3.58 (s, 3H).

3-(2-(*tert*-Butoxy)ethoxy)-5-methoxyaniline (95). To a solution of **94** (2.79 g; 20.5 mmol) in dry DMF (90 mL) cooled at 0 °C was added portionwise NaH (60% dispersion in oil, 1.76 g; 44.1 mmol). A solution of 2-(*tert*-butoxy)ethyl 4-methylbenzenesulfonate (5.46 g; 20.1 mmol) in

dry DMF (10 mL) was then added dropwise and the reaction mixture was stirred at rt overnight. Water was added and the reaction mixture was extracted with EtOAc. The organic phase was dried with Na_2SO_4, filtered and concentrated. Purification by flash column chromatography on silica gel using a gradient of EtOAc (5% to 50%) in heptane furnished 2.30 g (48%) of the title compound as a brown oil. ESI/APCI(+): 240 (M+H). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.96 (d, *J* = 2.4 Hz, 1H), 5.91 (t, *J* = 2.0 Hz, 1H), 5.88 (t, *J* = 2.0 Hz, 1H), 4.03 (t, *J* = 5.6 Hz, 2H), 3.75 (s, 3H), 3.70 (t, *J* = 5.6 Hz, 2H), 1.25 (s, 9H).

2-(3-amino-5-methoxyphenoxy)ethan-1-ol (96). A solution of **95** (0.400 g; 1.7 mmol) in a 4N HCl solution in dioxane (4.6 mL; 18.4 mmol) was stirred at rt overnight. The reaction mixture was basified with 2N NaOH and extracted with EtOAc. The organic phase was washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel using a gradient of MeOH (0% to 10%) in CH_2Cl_2 furnished 0.296 g (97%) of the title product as a white powder. ESI/APCI(+): 184 (M+H). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 5.72 – 5.80 (m, 2H), 5.68 (t, *J* = 2.0 Hz, 1H), 5.06 (s, 2H), 4.82 (t, *J* = 5.8 Hz, 1H), 3.84 (t, *J* = 5.2 Hz, 2H), 3.66 (t, *J* = 5.2 Hz, 2H), 3.63 (s, 3H).

Enantiomers separation of 100. Racemic **100** was subjected to chiral SFC purification (ChiralPak IC column; mobile phase: 60% CO_2, 40% MeOH) to provide **100a** and **100b**. **100a:** ESI/APCI(+): 417 (M+H). ESI/APCI(-): 415 (M-H). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.14 (br s, 1H), 8.90 (s, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.62 (br d, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.12 - 7.24 (m, 3H), 6.34 (d, *J* = 7.9 Hz, 1H), 5.99 – 6.12 (m, 3H), 5.70 (s, 1H), 4.79 (br s, 1H), 3.83 (dq, *J* =10.3, 5.1 Hz, 2H), 3.55 - 3.71 (m, 5H). Retention time: 1.9 min. ee > 99%. $[\alpha]_D^{20}$ = +39.7° (589 nm, c 0.292, DMF, 20°C). **100b:**

ESI/APCI(+):417 (M+H). ESI/APCI(-): 415 (M-H). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.15 (br s, 1H), 8.90 (s, 1H), 8.15 (br d, J = 7.6 Hz, 1H), 7.62 (br d, J = 7.6 Hz, 2H), 7.46 (br d, J = 7.9 Hz, 1H), 7.28 (br t, J = 7.4 Hz, 2H), 7.13 - 7.24 (m, 3H), 6.34 (br d, J = 8.2 Hz, 1H), 6.01 – 6.12 (m, 3H), 5.70 (s, 1H), 4.79 (br s, 1H), 3.77 – 3.90 (m, 2H), 3.55 - 3.70 (m, 5H). Retention time: 2.8 min. ee > 99%. $[\alpha]_D^{20}$ = -39.9° (589 nm , c 0.283, DMF, 20°C).

Biological methods.

Cells and virus. The following DENV strains were used: DENV-1 Djibouti strain D1/H/IMTSSA/98/606 (GenBank accession number AF298808; kindly provided by Dr. X. de Lamballerie, Aix-Marseille Université, Marseille, France), DENV serotype 2 (DENV-2) 'Rega' lab strain (kindly provided by Dr. V. Deubel, formerly at Institute Pasteur, Lyon, France), DENV-3 strain H87 (KU050695; kindly provided by Dr. X. de Lamballerie), DENV-4 DAKHD34460 (AF051107, only partial sequences available; kindly provided by Dr. X. de Lamballerie). DENV-2 was initially cultured on Vero cells (African Green monkey kidney cells, obtained from the European Collection of Cell Cultures [ECACC]), but later we switched to C6/36 mosquito cells (from *Aedes albopictus*; ATCC CRL-1660). All other DENV serotypes were grown in C6/36 cells. Vero cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% sodium bicarbonate. C6/36 cells were cultured in Leibovitz's L-15 medium (Thermo-Fisher Scientific) supplemented with 10% FBS, 1% nonessential amino acids (NEAA; Thermo-Fisher Scientific), 1% HEPES buffer (Thermo-Fisher Scientific), and 1% penicillin (100 U/mL)/streptomycin (100 µg/mL) solution (Thermo-Fisher Scientific) at 28 °C in the absence of CO_2.

Cytopathic effect (CPE)-reduction assay. Vero cells were seeded in 96-well plates at a density of 7×10^3 cells/well. One day later, cells were infected with DENV-2 'Rega' lab strain (MOI = 0.05) diluted in assay medium (MEM supplemented with 2% FBS, 0.075% sodium bicarbonate, and 2 mM L-glutamine) and in the presence of a 5-fold serial dilution of the compounds. After incubating for 7 days, the medium was removed and cells were fixed with ethanol and stained with a 1% methylene blue solution. For confirmation of hits, CPE-based assays were performed using the same protocol, but using a 2-fold serial dilution. Also, a newly synthesized batch for each potential hit compound was prepared.

Virus yield reduction assay. Vero cells were seeded in 96-well plates at a density of 5×10^4 cells/well. One day later, cells were infected with DENV (MOI = 0.01) diluted in assay medium. After one hour of incubation, input virus was removed and, after rinsing the cells 3 times, replaced with assay medium containing a 3- or 5-fold serial dilution of the compound. After an incubation period of 4 days (for DENV-2 and DENV-4) to 7 days (for DENV-1 and DENV-3), supernatant was harvested and the viral RNA load was determined by real-time quantitative RT-PCR, as described previously[27]. To determine a possible cytotoxic/-static effect of the compound, the same protocol was followed except that virus infection was omitted. Read out was performed using the MTS/PMS method (Promega), as described previously[28]. The 50% effective concentration (EC_{50}) and the 50% cytotoxic concentration (CC_{50}), which are defined as the compound concentration that is required to inhibit viral RNA replication by 50% respectively to inhibit cell growth by 50%, were determined using logarithmic interpolation.

Assay Interference Compounds

All compounds for biological testing were screened against known assay interference chemotypes[29] by the authors. No flags were reported.

Animals and Ethics

All experiments were performed in the on□site Association for Assessment and Accreditation of Laboratory Animal Care approved rodent facility according to the applicable animal welfare guidelines and legislation, and experimental procedures were approved by the institutional ethics committee.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

DENV, dengue virus; SI, selectivity index; MW, microwave irradiation; SAR, structure-activity relationships; HLM, human liver microsomes; RLM, rat liver microsomes; SFC, supercritical fluid chromatography.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website.

Experimental and analytical data of key intermediates.

Molecular Formula Strings with DENV-2 EC_{50} and CC_{50}.

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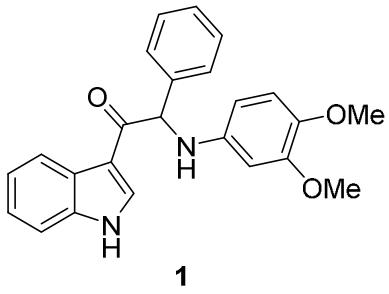


1

EC_{50} = 0.078 µM (DENV-2 'Rega' lab strain)
CC_{50} = 29 µM
SI = 380

Figure 1. Structure and properties of hit compound **1**

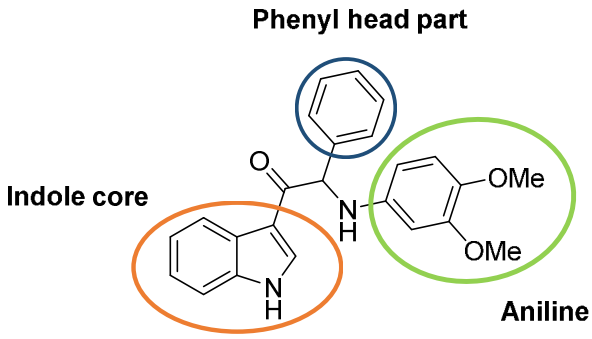
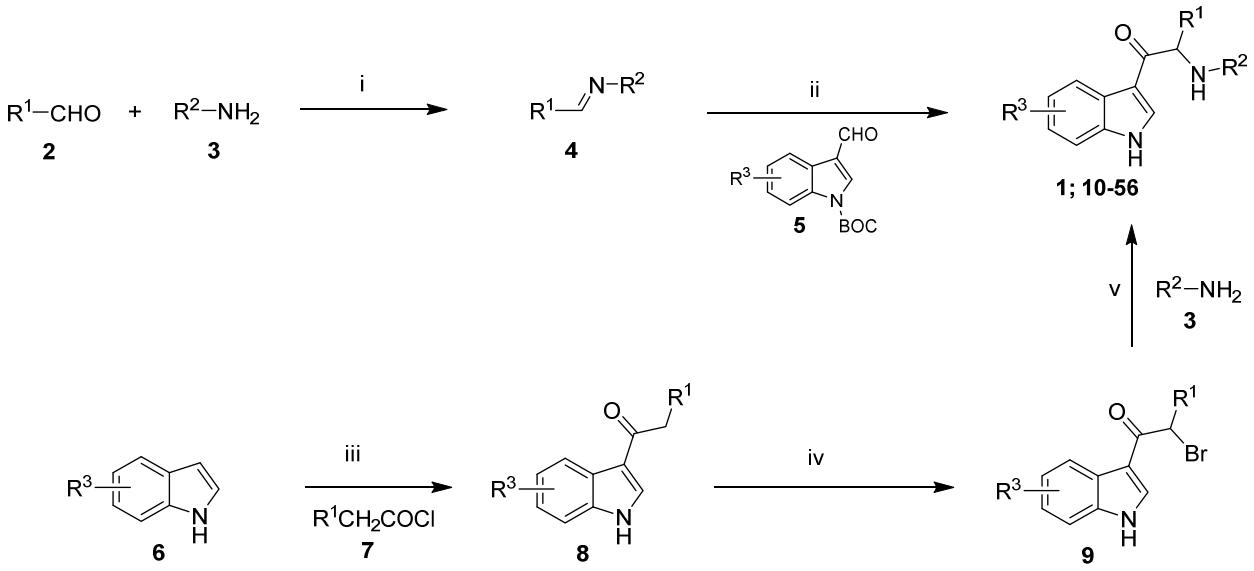


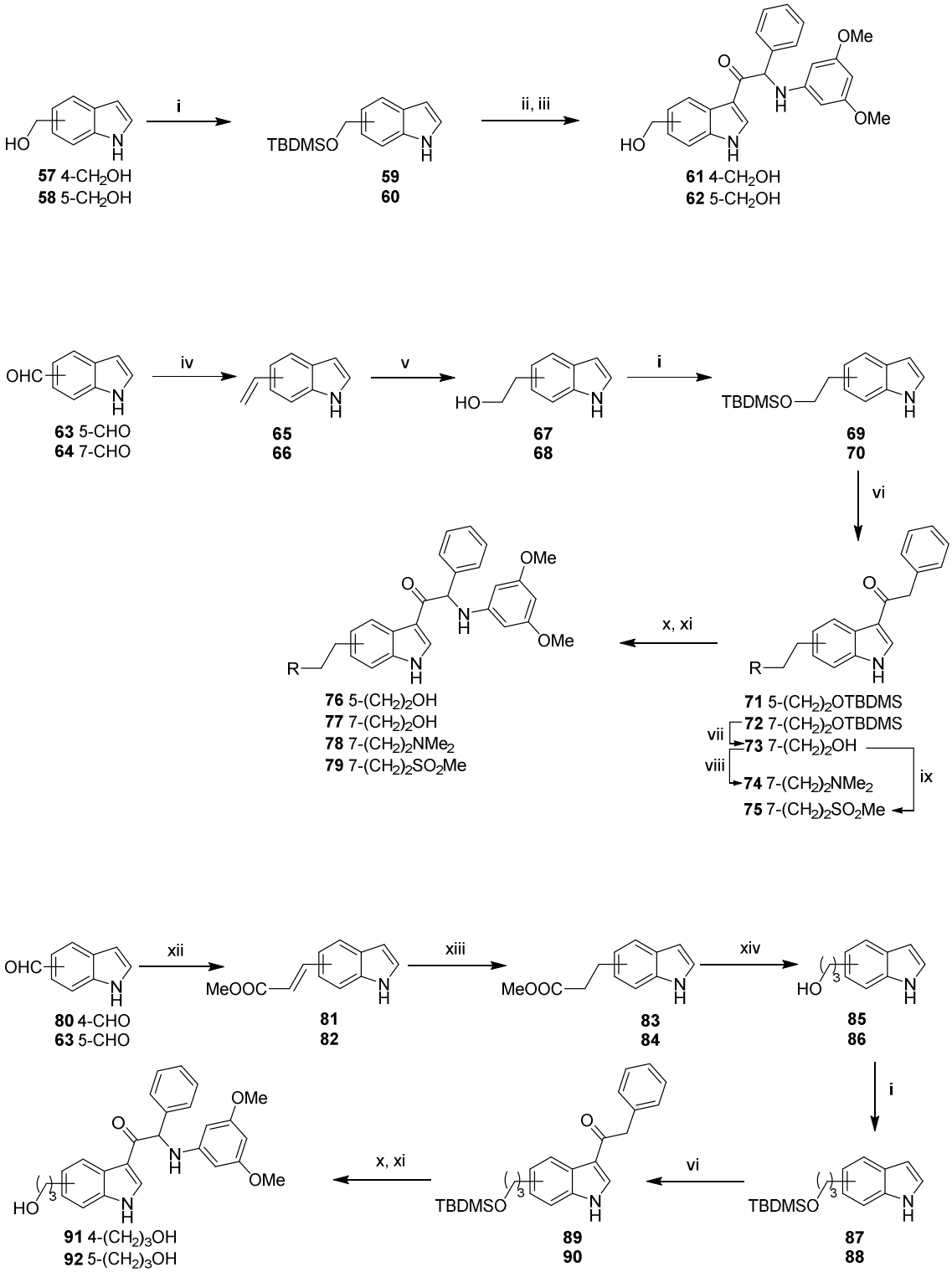
Figure 2. Decomposition of **1** in three structural subunits to explore SAR

Scheme 1. General synthetic routes to ketoindole derivatives[a]



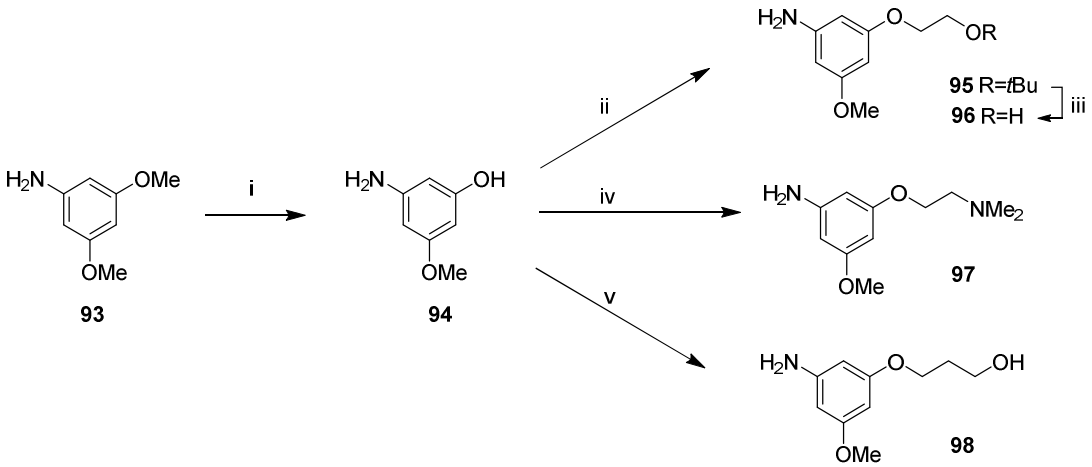
[a]Reagents and conditions: i) EtOH, 60 °C, 6 h; ii) a) 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium chloride, NEt_3, EtOH, 5 min, 70 °C; b) addition of **4** and **5**; c) 70 °C, overnight; d) MW irradiation, 160 °C, 4 min; iii) 1M Et_2AlCl, **7**, CH_2Cl_2, 0 °C, 3 h; iv) phenyltrimethylammonium tribromide, THF, 0 °C to rt, overnight; v) **3**, CH_3CN, MW irradiation, 100-150 °C, 10-30 min.

Scheme 2. Synthesis of derivatives with polar chains on indole[a]



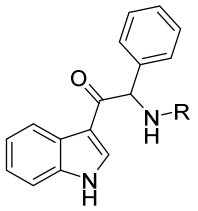
[a]Reagents and conditions: i) TBDMSCl, DBU, THF, rt, 4 h; ii) α-chlorophenylacetyl chloride, pyridine, toluene, 55 °C, 1 h; iii) 3,5-dimethoxyaniline, CH_3CN, MW irradiation, 130 °C, 15 min. iv) methyltriphenylphosphonium bromide, *t*BuOK, THF, 0 °C to rt, overnight; v) a) 1M BH_3.THF, THF, rt, 2 h; b) 30% H_2O_2, NaOH, rt, 4 h; vi) 1M Et_2AlCl, $PhCH_2COCl$, CH_2Cl_2, 0 °C, 2 h; vii) 4N HCl in dioxane, CH_2Cl_2, rt, 4 h; viii) a) MsCl, NEt_3, CH_2Cl_2, rt, 4 h; b) 2N Me_2NH, THF, 60 °C, 4 days; ix) a) MsCl, NEt_3, CH_2Cl_2, rt, 4 h; b) NaSMe, MeOH, rt, 3 days; c) *m*-CPBA, CH_2Cl_2, rt, 4 h; x) phenyltrimethylammonium tribromide, THF, 0 °C to rt, overnight; xi) substitution with 3,5-dimethoxyaniline; xii) $(EtO)_2P(O)CH_2COOMe$, K_2CO_3, THF, rt, overnight; xiii) H_2, Pd/C, MeOH, rt, overnight; xiv) $LiBH_4$, THF, EtOH, rt, overnight.

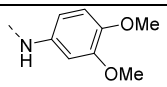
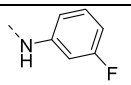
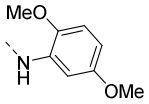
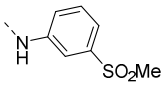
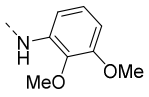
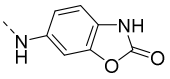
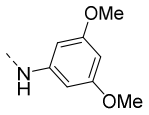
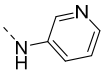
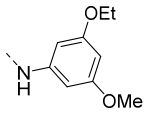
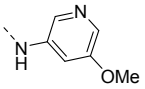
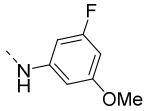
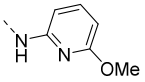
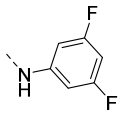
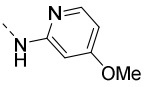
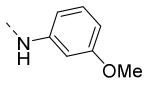
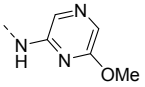
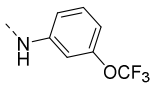
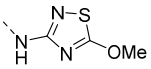
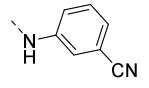
Scheme 3. Preparation of anilines with polar chains[a]



[a]Reagents and conditions: i) dodecanethiol, NaOH, NMP, MW irradiation, 160 °C, 1 h; ii) $TsOCH_2CH_2OH$, NaH, DMF, 0 °C to rt, overnight; iii) 4N HCl in dioxane, rt, overnight; iv) $MsOCH_2CH_2NMe_2$, NaH, DMF, 0 °C to rt, 60 h; v) $BrCH_2CH_2CH_2OH$, Cs_2CO_3, DMF, 60 °C, overnight.

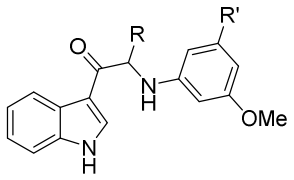
Table 1. Antiviral activity and cytotoxicity of compounds **1**, **10-28**

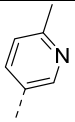
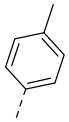
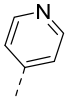
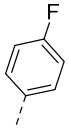
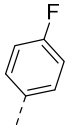
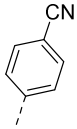
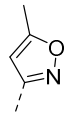
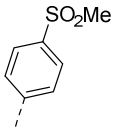
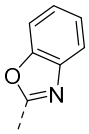
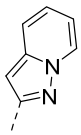
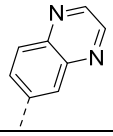


Cmpd		DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)	Cmpd		DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)
1		0.078	29	**19**		2.4	12
10		2.5	15	**20**		6.5	65
11		4.2	> 129	**21**		6.1	15
12		0.007	16	**22**		5.0	54
13		0.018	13	**23**		0.036	20
14		0.020	22	**24**		0.288	16
15		1.7	23	**25**		6.3	95
16		0.073	18	**26**		0.484	68
17		0.926	5	**27**		3.3	90
18		2.1	14	**28**		15	86

[a]DENV-2 'Rega' lab strain. EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR. CC_{50} values were determined by colorimetric read out (i.e. MTS/PMS).

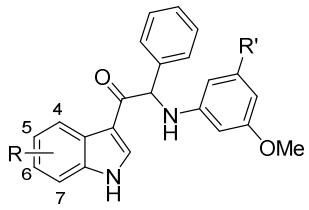
Table 2. Antiviral activity and cytotoxicity of compounds **16**, **29-47**



Cmpd	R	R'	DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)	Cmpd	R	R'	DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)
16		H	0.073	18	**38**		H	0.073	73
29		H	0.022	20	**39**		H	0.138	16
30		H	0.269	20	**40**		H	0.391	100
31		H	0.096	14	**41**		H	0.837	65
32		H	0.025	14	**42**		H	0.134	15
33		OMe	0.004	13	**43**		H	0.297	16
34		H	0.041	14	**44**		H	0.590	50
35		H	1.6	> 115	**45**		H	0.011	13
36		H	0.138	71	**46**		H	0.028	26
37		OMe	0.015	69	**47**		H	1.2	19

[a]DENV-2 'Rega' lab strain. EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR. CC_{50} values were determined by colorimetric read out (i.e. MTS/PMS).

Table 3. Antiviral activity and cytotoxicity of compounds **16**, **48-56**



Cmpd	R	R'	DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)
16	H	H	0.073	18
48	4-Cl	H	0.024	18
49	4-F	H	0.122	21
50	5-F	H	0.034	13
51	5-F	OMe	0.007	13
52	6-F	H	0.045	15
53	6-Cl	H	0.013	13
54	6-OMe	H	0.043	13
55	7-F	H	0.057	14
56	7-Me	H	0.051	> 135

[a]DENV-2 'Rega' lab strain. EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR. CC_{50} values were determined by colorimetric read out (i.e. MTS/PMS).

Table 4. Antiviral activity and cytotoxicity of racemic compound **12** and its enantiomers

Cmpd		DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)
12	racemic	0.007	16
12a	(+)-enantiomer	0.001	13
12b	(-)-enantiomer	0.348	11

[a]DENV-2 'Rega' lab strain. EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR. CC_{50} values were determined by colorimetric read out (i.e. MTS/PMS).

Table 5. Evaluation of broad-spectrum activities for a selection of compounds

Strain	DENV-1 Djibouti	DENV-2 'Rega' lab strain	DENV-3 H87	DENV-4 Dakar
Cmpd	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)
1	1.6	0.078	29.4	4.5
12a	0.047	0.001	0.092	1.9
37	0.173	0.015	0.310	2.5
51	0.247	0.007	0.680	4.8

EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR.

Table 6. ADMET properties for a selection of compounds

Compound	**12**	**12a**	**12b**	**23**	**45**	**100a**
Solubility[a] (μM)	9	6	9	42	1	48
Permeability[b]						
P_{app} A-B (-GF)[c] (10^{-6} cm.s^{-1})	7.5	7.6	7.4	4.7	5.6	1.8
P_{app} A-B (+GF)[d] (10^{-6} cm.s^{-1})	8.5	8.4	9.0	9.7	5.7	7.9
HPPB[e] (% bound)	99.8	99.7	99.9	99.5	> 99.8	99
RPPB[f] (% bound)	99.9	99.8	> 99.9	99.2	> 99.6	99.2
HLM (% metabolized)[g]	11	14	16	23	19	2
RLM (% metabolized)[h]	63	37	91	86	99	21
hERG IC_{50} (μM)	> 50	ND[i]	ND	3.5	ND	> 50
CYP IC_{50} (μM)						
CYP1A2	2.4	1.4	6.4	2.8	4.2	> 10
CYP2C19	4.0	2.3	3.0	2.5	3.2	4.4
CYP2C9	4.8	3.2	3.0	3.3	6.1	3.1
CYP2D6	> 10	> 10	> 10	8.4	> 10	> 10
CYP3A4	> 10	> 10	> 10	0.05	> 10	> 10

[a]At pH = 7.4 from DMSO solution. [b]MDR1 transfected LLC-PK1 cells. [c]Permeability assay performed in the absence of a P-gp inhibitor (GF120918). [d]Permeability assay performed in the presence of a P-gp inhibitor (GF120918) . [e]Human protein plasma binding. [f]Rat protein plasma

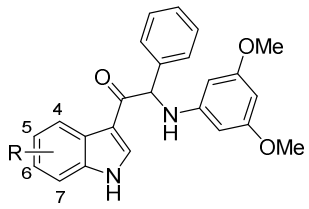
binding. [g]% Metabolized in human liver microsomes after 15 minutes. [h]% Metabolized in rat liver microsomes after 15 minutes. [i]Not determined.

Table 7. Pharmacokinetic parameters following intravenous and oral administration in rats

Cmpd	iv PK parameters[a]				Oral PK parameters[b]			
	Dose (mg/kg)	CL (mL/min/kg)	Vss (L/kg)	$T_{1/2}$ (h)	Dose (mg/kg)	Cmax (nM)	AUC last (nM.h)	F (%)
12a	2.5	67	26	4.7	10	88	422	7
100a	2.5	35	5.8	2.4	10	706	7323	64

[a]Formulation for iv dose: PEG400/water 7/3. [b]Formulation for oral dose: PEG400.

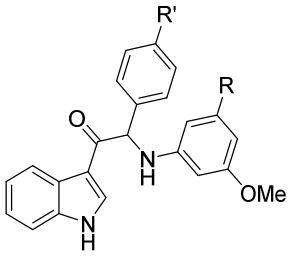
Table 8. Properties of compounds with polar chains on indoles



Cmpd	R	DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)	Solubility (μM)	HLM $t_{1/2}$ (min)
12	H	0.007	16	9	> 60
61	4-CH_2OH	0.288	71	ND[b]	ND
91	4-$(CH_2)_3OH$	1.4	55	ND	ND
62	5-CH_2OH	0.106	65	ND	ND
76	5-$(CH_2)_2OH$	0.181	56	ND	ND
92	5-$(CH_2)_3OH$	0.096	56	23	38
77	7-$(CH_2)_2OH$	0.093	19	< 1	36
78	7-$(CH_2)_2NMe_2$	2.0	12	ND	ND
79	7-$(CH_2)_2SO_2Me$	0.406	> 102	ND	ND

[a]DENV-2 'Rega' lab strain. [b]Not determined. EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR. CC_{50} values were determined by colorimetric read out (i.e. MTS/PMS).

Table 9. Properties of compounds with polar chains on anilines



Cmpd	R	R'	DENV-2[a] EC_{50} (μM)	MTS CC_{50} (μM)	Solubility (μM)	HLM (% met.)[b]	RLM (% met.)[b]
12	OMe	H	0.007	16	9	11	63
99	CH_2OH	H	0.065	71	50	18	85
100	$O(CH_2)_2OH$	H	0.059	47	60	13	37
100a (+)-enantiomer	$O(CH_2)_2OH$	H	0.012	105	48	2	21
100b (-)-enantiomer	$O(CH_2)_2OH$	H	0.456	77	ND[c]	ND	ND
101	$O(CH_2)_2OH$	F	0.013	46	27	7	23
102	$O(CH_2)_2NMe_2$	H	1.7	13	ND	ND	ND
103	$O(CH_2)_3OH$	H	0.085	50	30	8	67

[a]DENV-2 'Rega' lab strain. [b]%Metabolized after 15 minutes.[c]Not determined. EC_{50} values were determined in virus yield reduction assays, followed by RT-qPCR. CC_{50} values were determined by colorimetric read out (i.e. MTS/PMS).

Insert Table of Contents Graphic and Synopsis Here