I. MECHANISTIC STUDIES OF ANTI-MALARIAL SPIROINDOLONES

AND

II. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF AN INHIBITOR OF DENGUE PROLIFERATION

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NATIONAL UNIVERSITY OF SINGAPORE

UNIVERSITY OF BASEL

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UNIVERSITY OF BASEL

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To the people mentioned above, I wish you all the best and may you stay healthy and live life to its fullest!

VOLUME I:

MECHANISTIC STUDIES OF ANTI-MALARIAL SPIROINDOLONES

Table of Contents (Volume I)

Summary

NITD20, a member of indoline-spiro-tetrahydro-β-carboline class of compounds, was identified as a powerful inhibitor of *Plasmodium falciparum* proliferation. Synthetic studies in our laboratory showed that the synthesis of this compound exhibits high diastereoselectivity. This study investigated the reaction mechanism involved. Imine intermediates were synthesized, characterized and further cyclized at different temperatures to obtain indoline-spiro-tetrahydro-β-carbolines of different diastereoselectivities. Control of the stereochemistry of the indoline-spiro-tetrahydro-β-carboline was demonstrated and a hypothesis for the mechanism of the reaction will be presented.

List of Tables

List of Figures

1. Introduction

1.1. Malaria and its treatment

Malaria is an infectious disease caused by *Plasmodium* parasites, which are transmitted by *Anopheles* mosquitoes. The *Plasmodium* life cycle consists of several transitions and stages. A bite by the infected *Anopheles* mosquitoes will release sporozoites into the host to initiate a liver stage infection. After about a week, the infected hepatocytes will rupture and release merozoites which will move on to invade erythrocytes. The parasites will mature within 2-3 days and eventually turn to gametocytes that will be ingested by another *Anopheles* mosquito taking its next blood meal (1). Among the four *Plasmodium* species, namely *falciparum*, *vivax*, *ovale* and *malariae*, that cause malaria in humans, *Plasmodium falciparum* is the most virulent and is responsible for the majority of deaths from malaria. *Plasmodium vivax* and *ovale* are less deadly but the ability of these parasites to stay dormant in the liver makes them difficult to be resolved in the host. Similarly, *Plasmodium malariae* can exists as an asymptomatic blood stage infection for decades in the host (2). Clinical symptoms of malaria develop within 2-6 weeks of an infective bite and include fever and general weakness for uncomplicated cases and coma, pernicious anemia and pulmonary edema for complicated cases (3).

In 2006, there were approximately 247 million malaria cases among 3.3 billion people at risk in the world. These infections translated to nearly a million deaths, mostly of children under the age of 5. In 2008, 109 countries were endemic for malaria and the disease is most prevalent in Africa (4). Under the Millennium Development Goals, a target was set to halt the rising incidence of malaria by 2015 (5). This rising awareness has led to an increase in the research efforts for this tropical disease over the recent years and progress in the areas of chemotherapies, vaccines, and diagnostics have raised hopes for delivery of interventions that will reduce the burden of this disease (2).

Historically, there is a long list of chemotherapeutic agents used for the treatment of malaria. In 1820, Pierre-Joseph Pelletier and Joseph Bienaimé Caventou extracted an alkaloid from the cinchona bark and named it quinine, giving rise to the world's first effective treatment for malaria (6). Being extremely bitter and not well tolerated by patients when taken orally, quinine was soon replaced. When the WHO launched the Global Malaria Eradication Programme in 1955, chloroquine became the treatment of choice instead (7). However, chloroquine resistance was detected after several decades of use and drugs such as the sulfadoxine-pyrimethamine combination, proguanil, mefloquine and atovaquone (Figure 1) were subsequently introduced between the 1950s and 1990s to dampen the effects of chloroquine resistance. The emergence of resistance to these newer drugs proved to be even faster than chloroquine as drug resistance emerged after several years of use. Artemisinin (Figure 1), a natural product extracted from *Artemisia annua*, and its derivatives remain the only anti-malarial left with high potency and low resistance (2). Currently, artemisinin-based combination therapy (ACT), a combination of artemisinin derivatives with other active anti-malarials, is the most effective form of treatment against malaria (8). Studies have shown that using two or more drugs in combination has the potential to delay the development of resistance, (9) which explains the present adoption of combination therapies for the management of malaria. For example, Coartem, developed by Novartis and Chinese partners in 1994, is a combination of artemether and lumefantrine (Figure 1). To date, more than 6 million patients have benefited from this treatment since its first registration in October 1998 (10). The combination of sulfadoxine and pyrimethamine is also commonly employed in the treatment of malaria in pregnant women. And in the event of severe multi-drug resistant malaria, quinine taken in combination with other antibiotics, such as doxycycline and clindamycin, is still an adequate treatment (8). However, the few current treatments of malaria are vulnerable to failure once compound-resistant parasites emerged. Thus, the lack of new anti-malarials in the clinical setting against different stages of the parasite is a serious threat for the treatment of malaria in the future. This has spurred research efforts on the

development of more ACTs and the discovery of novel anti-malarials, which are more potent, faster acting, minimally toxic and have chemical scaffolds different from the drugs in use.

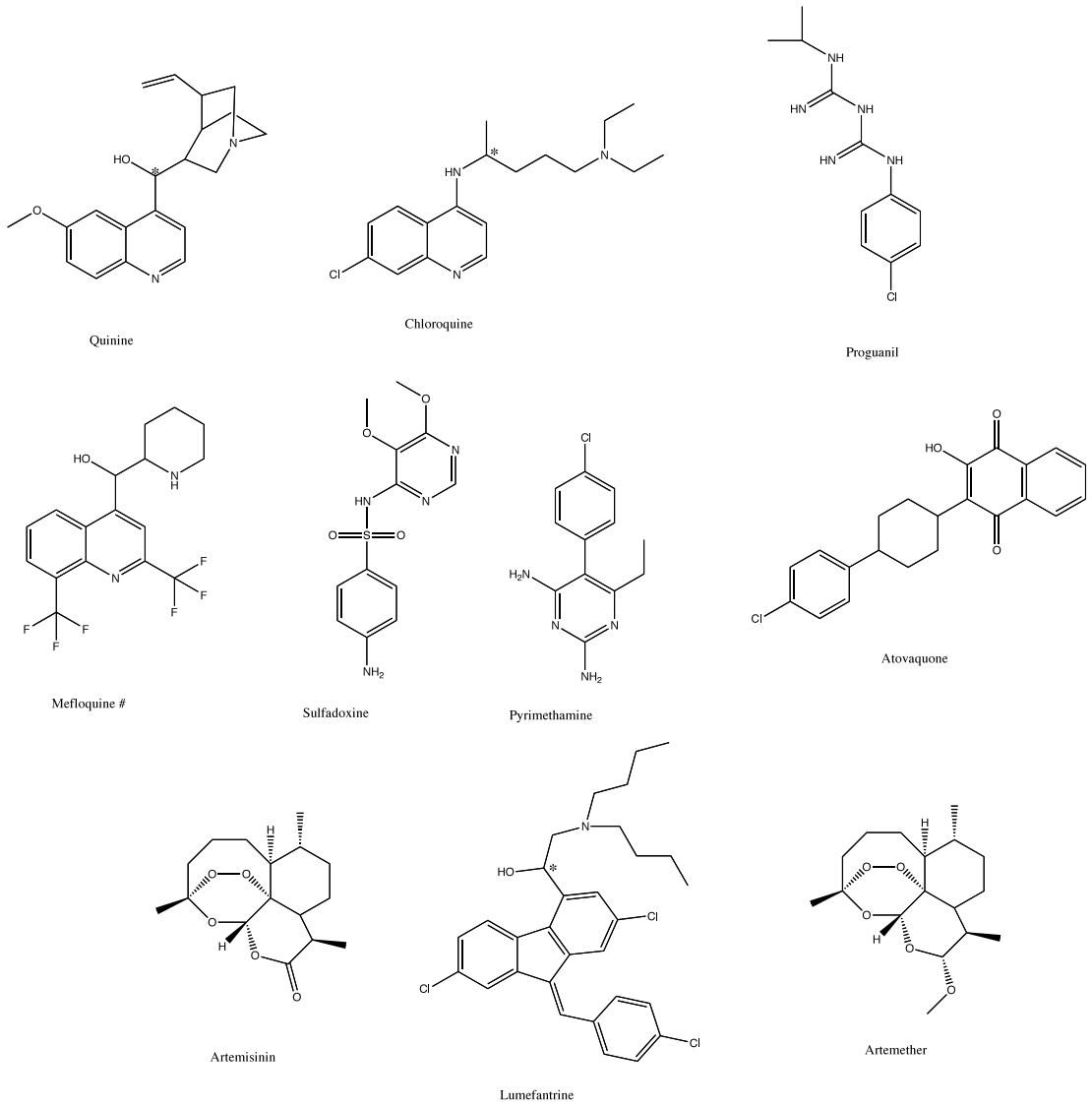


Figure 1 Structures of some common anti-malarials (* indicates a racemate; #mefloquine is a mixture of diastereomers)

1.2. Screening and identification of potent growth inhibitor of *Plasmodium falciparum*

At the Novartis Institute for Tropical Diseases (NITD), research efforts are directed towards finding a single dose cure for malaria caused by *Plasmodium falciparum*. After screening

a library of natural products with over ten thousand members for activity in a high-throughput cellular proliferation assay, NITD20 was identified as a powerful inhibitor of the parasite's proliferation with a good pharmacological profile. This compound has a novel chemical scaffold (Figure 2) as compared to existing anti-malarials and its indoline-spiro-tetrahydro-β-carboline structure has two chiral centers. Re-synthesis of NITD20 via the Pictet-Spengler reaction yielded the product with high diastereoselectivity. After purification by column chromatography, NITD20 was isolated and both the relative and absolute stereochemistry of the compound were determined by X-ray crystallography (Figure 3).

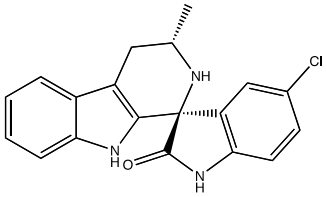


Figure 2 Structure of NITD20

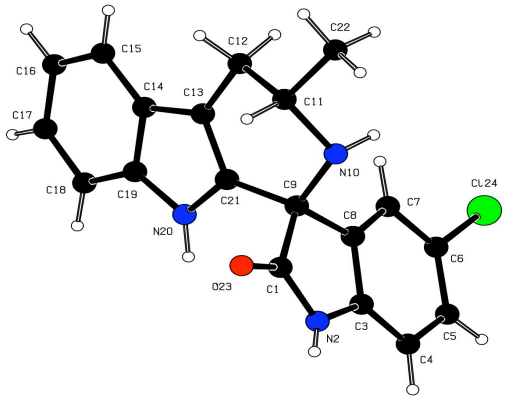


Figure 3 X-ray crystal structure of NITD20 showing the crystallographic numbering of the atoms

Intrigued by the mechanism behind the synthesis of NITD20, we sought a better understanding of the high diastereoselectivity observed with this spiro-tetrahydro-β-carboline compound.

1.3. The Pictet-Spengler reaction and control of its diastereoselectivity

Amé Pictet and Theodor Spengler first discovered the Pictet-Spengler reaction in 1911 when they condensed phenethylamine with dimethoxyethane to produce tetrahydroisoquinoline (11). The reaction, which occurs in plants and humans, is now one of the most direct methods of forming the tetrahydro-β-carboline ring system (Figure 4), which is commonly found in indole and isoquinoline alkaloids. For example, the biosynthesis of indole-derived natural products utilizes the enzyme-mediated Pictet-Spengler reaction of tryptophan, tryptamine or dopamine with naturally occurring aldehydes as an essential step in the construction of the carbon skeleton (12). An example of such a Pictet-Spengler reaction is found in the biosynthesis of strychnine.

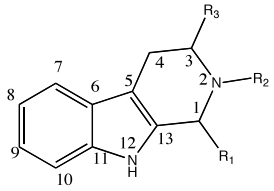


Figure 4 Structure of a tetrahydro-ß-carboline

There are two possible pathways (Figure 5) for the reaction to proceed (13). A common imine intermediate is formed initially and attack at imine **a** can occur either directly via route 1 to form the pentahydro-β-carboline carbonium ion **c** or via route 2 followed by a rearrangement of the spiroindolenine **b**. With the help of an isotopic labeling experiment, Bailey et al. (14) demonstrated the formation of 3-aza-tetrahydro-β-carboline, from hydrazine and methanal in aqueous conditions, via the symmetrical spiro intermediate **b** (Figure 5). In this experiment, the authors hypothesized that if the attack at imine **a** occurred via route 1, the isotopic label would be localized at C1 of product **d**, whereas if the attack occurred via route 2, the label would be distributed between C1 and C4 of the product. The latter was achieved and the formation of the spiro intermediate was confirmed. Since imine formation is reversible in the presence of water, the formation of this spiro intermediate was determined to be fast and reversible and would not be

expected to affect the stereochemistry of the final tetrahydro-β-carboline product in a standard Pictet-Spengler reaction. On the other hand, the formation of the carbonium ion **c** was deduced to be the slow rate-determining step and energies of the *cis* and *trans* transition states should control the stereochemistry of the final product. In particular, for compounds with substituents at C1 and C3 of the tetrahydro-β-carboline ring structure (Figure 4), the Pictet-Spengler reaction can be used to establish the tricylic ring system with control of the stereochemistry at the chiral centers C1 and C3.

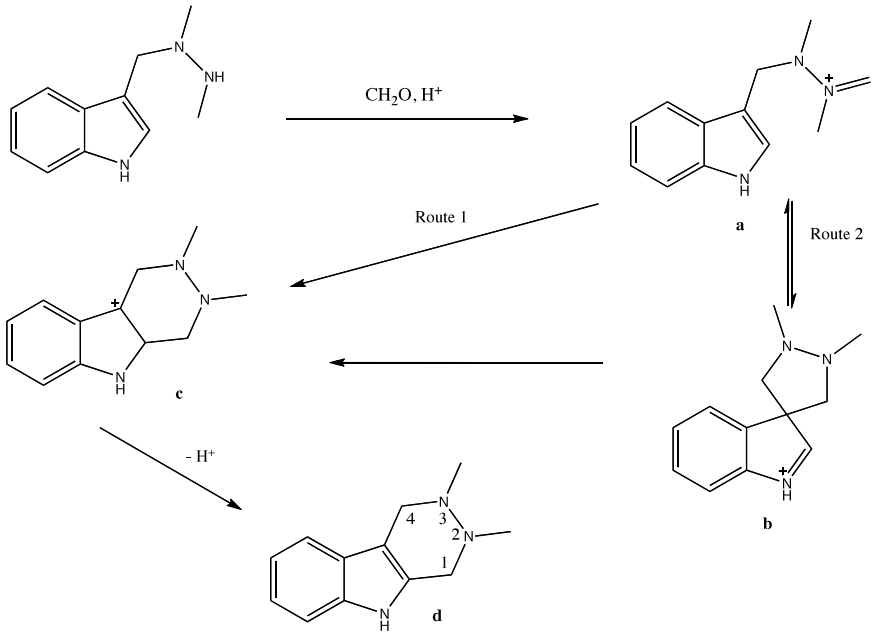


Figure 5 Mechanism for the formation of the 3-aza-tetrahydro-β-carboline

Over the recent years, the stereospecific Pictet-Spengler reaction has seen many developments. Alberch et al. (15) reported the synthesis of *cis*-tetrahydro-β-carbolines via the Pictet-Spengler reaction of tryptophan allyl ester with aryl aldehydes under kinetically controlled conditions. Results showed that only allyl esters led to *cis*-stereospecificity in the reaction while propyl ester gave a mixture of diastereomers. Similarly, only aryl aldehydes condensed *cis*-stereospecifically and alkyl aldehydes gave a mixture of diastereomers instead. A hypothesis that

favorable π-stacking interactions between the allyl and aryl groups allowed the cyclization to proceed through a di-axial intermediate leading to the formation of the *cis* diastereomer was put forward (Figure 6). Stabilization offered by π-stacking was postulated to overcome the steric strain associated with the di-axial conformation, thus forming the *cis* diastereomer.

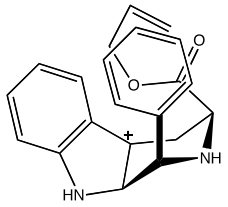


Figure 6 Proposed π-stacking between the allyl and aryl group in a di-axial intermediate

In another report, Ducrot et al. (16) were able to prepare diastereomerically pure tetrahydro-β-carbolines from reaction of α-aminoaldehydes with tryptamine. Experimental results showed that the use of bulky amino-protecting groups, led to the *trans* configuration while the use of smaller protecting groups led to the *cis* configuration. Furthermore, the *cis* diastereomer was formed exclusively under kinetic conditions. Under thermodynamic conditions, the diastereoselectivity was reversed and the amount of *trans* diastereomer formed was increased. The mechanism by which the *cis* and *trans* diastereomers may possibly interconvert under acid-catalyzed thermodynamic conditions is shown in Figure 7 (17).

Cox et al. (17) highlighted the influence of conformational effects on the diastereoselectivity of the Pictet-Spengler reaction. As illustrated in Figure 8, the spiroindolenine intermediate **a** resulting from attack 1 was less sterically hindered than **c** generated from attack 2. Subsequent rearrangement of the spiroindolenine intermediate along route 1 gave a more stable pentahydro-β-carboline carbonium ion **b** than **d** observed along route 2 because in **b**, the equatorial position occupied by the N-substituted phenyl group is more favorable than the axial position observed in **d**. These factors favored the generation of the *trans* diastereomer via route 1.

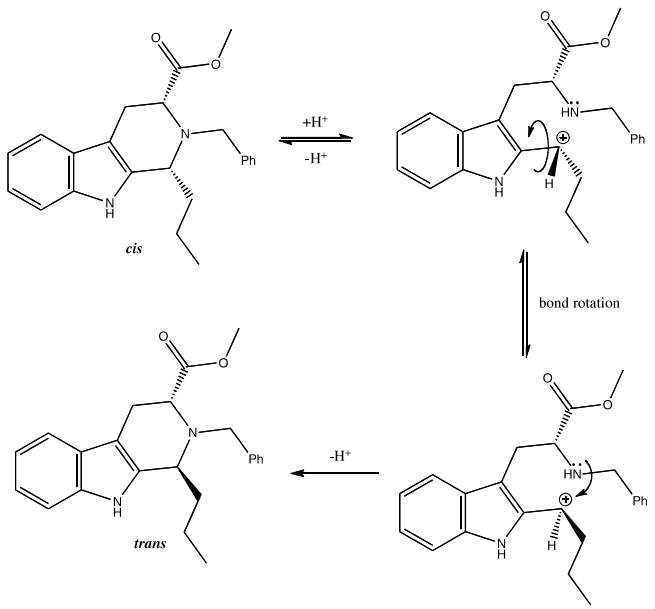


Figure 7 Proposed mechanism for the inter-conversion between the *cis* and *trans* configuration

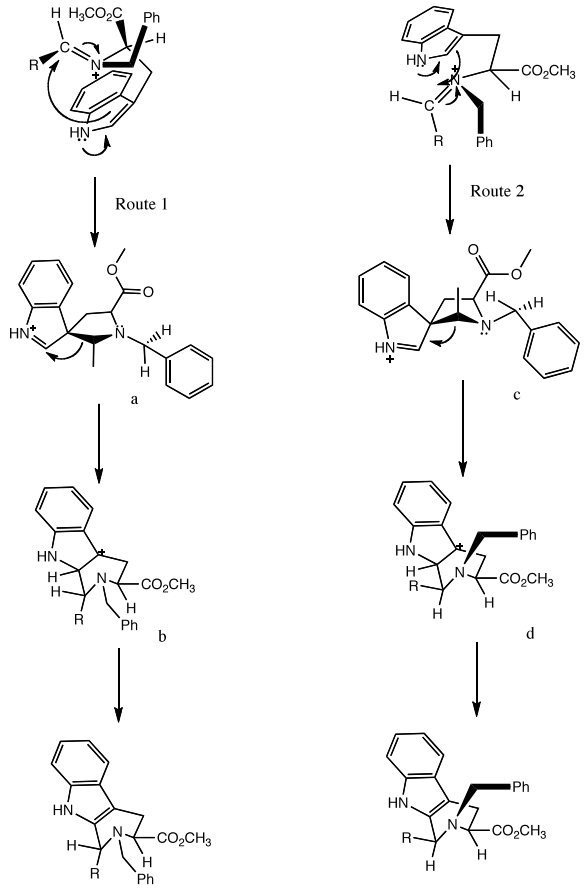


Figure 8 Different configuration of the imine intermediate yield different diastereomer

Among the different mechanisms suggested by several groups, the one (13) proposed by Bailey et al. was seen to be the most relevant for this study. As shown in Figure 9, the common imine intermediate formed initially could be attacked via route 1 or 2. It has been shown that route 2 does occur but is not rate-determining and would not be a source of stereochemical control in the Pictet-Spengler reaction. Formation of the carbonium ion **c** was inferred to be rate determining instead. In this study, both routes would be taken into consideration for the discussion of the stereochemical outcome of the reaction.

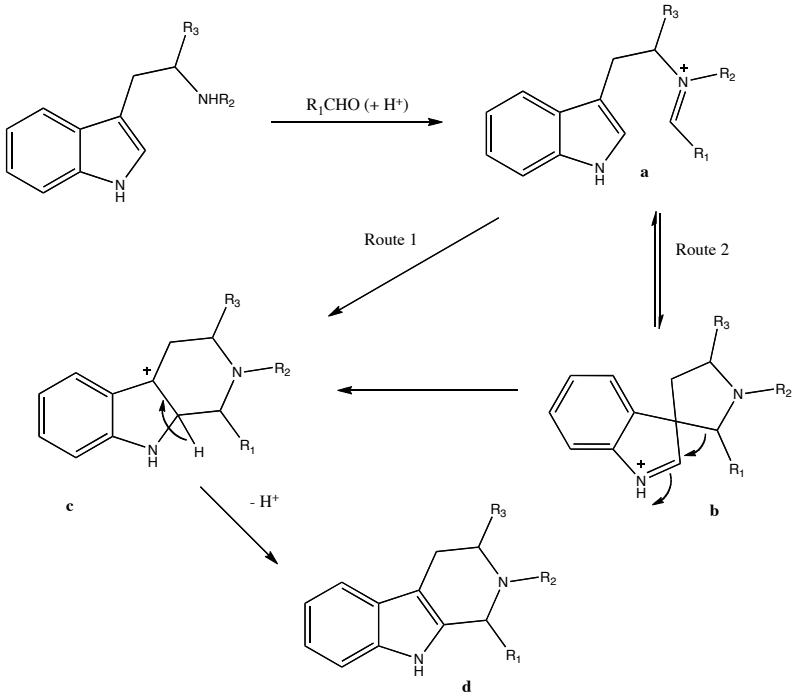


Figure 9 Possible mechanisms for the Pictet-Spengler reaction

1.4. Pictet-Spengler reaction between methyl tryptamine and 5-chloroisatin

The above-mentioned examples analyzed the tetrahydro-β-carboline structure for factors affecting the diastereoselectivity of the Pictet-Spengler reaction. However, limited information is available on the factors influencing the diastereoselectivity of the indoline-spiro-tetrahydro-β-carboline system found in our compound of interest, NITD20. Pogosyan et al. reported the

synthesis of similar indoline-spiro-tetrahydro-β-carboline derivatives but provided no information on the mechanism and diastereomeric ratio (18). Kuo et al. highlighted the usage of microwave irradiation to accelerate the Pictet-Spengler reaction between tryptophan and ketones to give 1,1-disubstituted tetrahydro-β-carbolines, but did not discuss the stereochemistry of the compounds or mechanism of the reaction (19). An attempt was made to elucidate the mechanism of the Pictet-Spengler reaction responsible for the high diastereoselectivity observed during the synthesis of NITD20. Furthermore, emphasis was placed on determining the influence of the configuration of the imine intermediate on the diastereoselectivity of the Pictet-Spengler reaction.

The synthesis of NITD20 commenced from the reaction between *S* methyl tryptamine and 5-chloroisatin as shown in Figure 10. The reaction was refluxed at 110ºC with *para*-toluenesulfonic acid as catalyst and ethanol as solvent. This set of conditions yielded NITD 20 (*trans* diastereomer) as the major product and the *cis* diastereomer as the minor product and the diastereomeric ratio was determined to be 7:1 by HPLC.

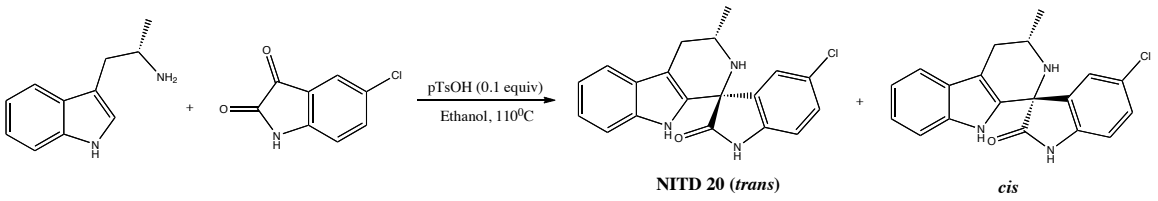


Figure 10 Synthesis of NITD20

For the mechanistic studies presented in this thesis, the racemic methyl tryptamine, which was synthetically more accessible, was used instead of the chiral methyl tryptamine. The chirality of methyl tryptamine will have no influence on the results as the diastereoselectivity was investigated and not the enantioselectivity. Using racemic methyl tryptamine, four structures could possibly be formed as illustrated in Figure 11. They are the two pairs of diastereomers (*trans/cis)* and the two pairs of enantiomers (*cis/cis* and *trans/trans).* The relative stereochemistry of all the products was assigned based on NMR and available X-ray crystallographic information.

The *cis* configuration is defined as having the two stereocenters on the ring in the same relative configuration while the *trans* configuration is defined as having the two stereocenters on the ring in the opposite relative configuration.

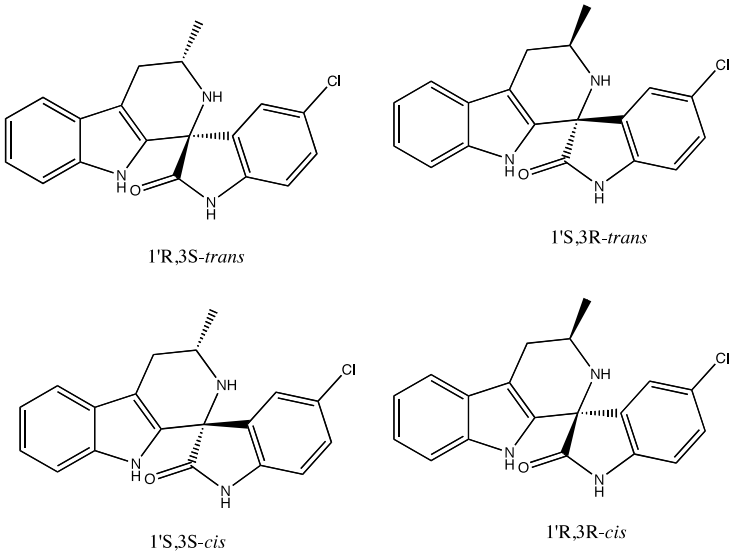


Figure 11 Possible structures for *trans* and *cis* products

The stability of the imine intermediates, the possibility of isomerization of the imines and the role of the imine configuration in governing the final stereochemistry of the indoline-spiro-tetrahydro-β-carboline structure shown in Figure 10 remained uncertain. In order to obtain a better understanding of the importance of these factors, the isolation, study and full characterization of the imine intermediates were necessary.

2. Results & Discussion

Abadi et al. reported the synthesis of 2-indolone imine derivatives by condensation of isatin or haloisatin with amino acids or histamine (20). For example, histamine was condensed with 5-chloroisatin in refluxing ethanol at 1M concentration. Given the similarity between the imine derivatives synthesized by the authors and the imine intermediates in this study, the synthetic method was adopted. However, attempts to reproduce the reported results provided the cyclized product **7** instead of the imine derivative (Figure 12). In these attempts, the imine derivative **a** could not be obtained.

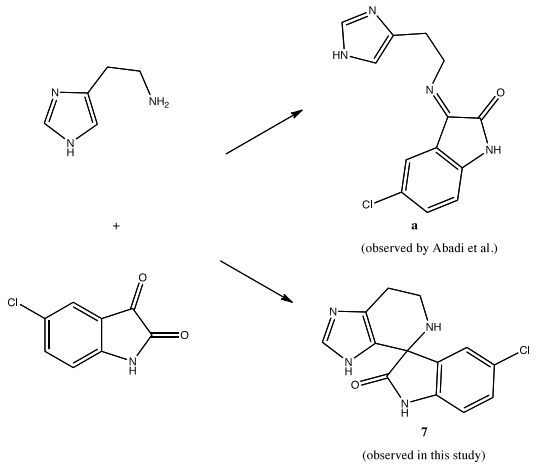


Figure 12 Different results obtained from the condensation of histamine and 5-chloroisatin

2.1. Investigation of imines formed between methyl tryptamine and 5-chloroisatin

2.1.1. Synthesis and characterization of imines 1 and 2

After some modifications to the procedure described by Abadi et al., the synthesis of the imine intermediates was successful. Imines **1** and **2** were obtained as mixtures of *Z*/*E* isomers from methyl tryptamine and 5-chloroisatin (Figure 13). The ratio of each mixture was determined, without purification, by comparing the integrated intensities of the methyl protons k in ^{1}H NMR

and expressed as *Z*:*E*. An example of the difference in integration of methyl protons k of both isomers is illustrated in Figure 14.

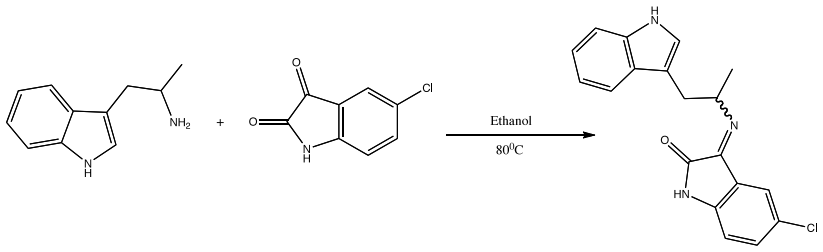


Figure 13 Synthesis of imine intermediate

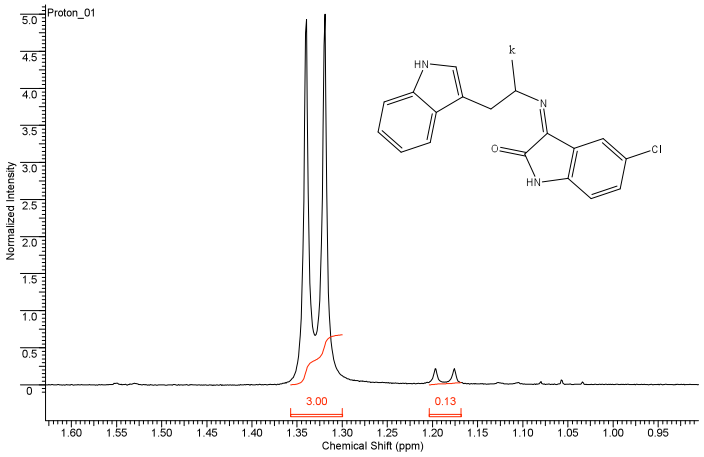


Figure 14 Integration of methyl protons k of both isomers

When methyl tryptamine was reacted with 5-chloroisatin in refluxing ethanol (80ºC) at 0.95M concentration, a 1:8 isomer mixture was first isolated serendipitously as the solvent evaporated in the midst of the reflux and a yellow precipitate remained. When the same experiment was repeated in a sealed tube to prevent evaporation of the solvent, a pale yellow precipitate was obtained instead. Analysis of this solid revealed a 20:1 isomer mixture, in contrast to the 1:8 mixture obtained under the previous conditions. This 20:1 isomer mixture was reproducible under the conditions of 80ºC ethanol at 0.95M concentration, fully characterized and named imine **1** (58% yield).

In an attempt to obtain the 1:8 isomer mixture again, the reaction mixture, at 0.95M concentration in 80ºC ethanol, was left to evaporate on purpose and a bright yellow solid was obtained as a 1:23 isomer mixture. It was hypothesized that due to the evaporation of ethanol, the 1:23 isomer mixture was precipitated when the reaction mixture was more concentrated than 0.95M. To test this hypothesis, two experiments were performed, where methyl tryptamine and 5-chloroisatin were reacted in a sealed tube under 80ºC ethanol at 1.45M or 2.87M concentration, and the ratio of the isomer mixture obtained was 2:1 and 1:23 respectively. This phenomenon of different reaction concentrations providing distinct isomer mixtures has few or no precedents in the literature and is difficult to explain. It probably involves a complex reaction in which solubility and equilibration of imines each play a role in determining the final ratio of the isomer mixture and more research has to be done to explain these observations. Nonetheless, the set of reaction conditions, which yielded the 1:23 isomer mixture as a bright yellow precipitate, was reproducible and allowed the characterization of the isomer mixture. The 1:23 isomer mixture was named imine **2** (58% yield).

Although characterization of both imines **1** and **2** by ^{1}H NMR revealed a distinct set of chemical shifts for each isomer, the differentiation of isomers was not clear during the initial phase of this study. For example, the chemical shifts of the two isomers of proton j have a difference of about 1 ppm (Figure 15). Proton j of the *Z* isomer was predicted to appear more upfield in the ^{1}H NMR spectra as it was thought to lie in a less electron-shielded position as compared to that of the *E* isomer.

Eventually, the two isomers were distinguished from each other by NOESY-1D spectroscopy. Irradiation of proton j (refer to Figure 16 for nomenclature) was deduced to show a correlation with proton f in the *E* isomer while in the *Z* isomer, no correlation between protons j and f was expected. Experimental results confirmed these predictions.

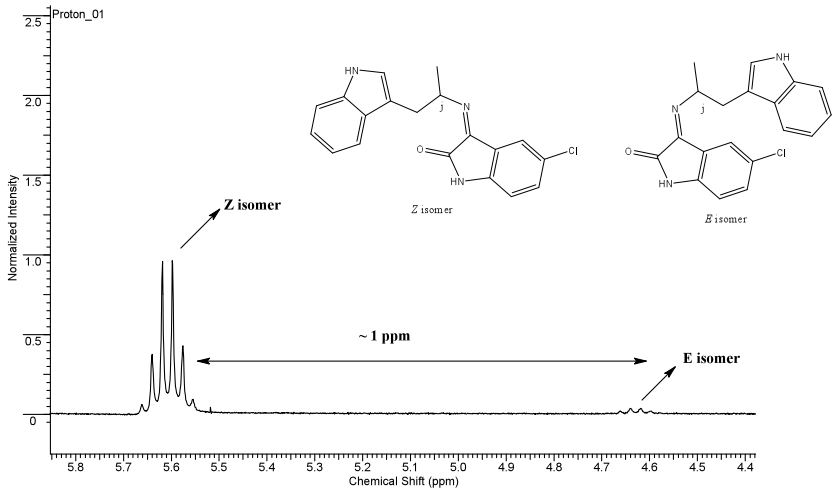


Figure 15 Chemical shifts of both isomers of proton j

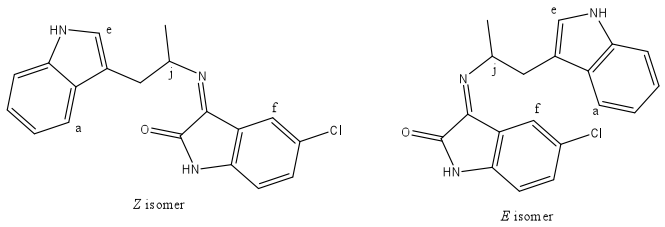
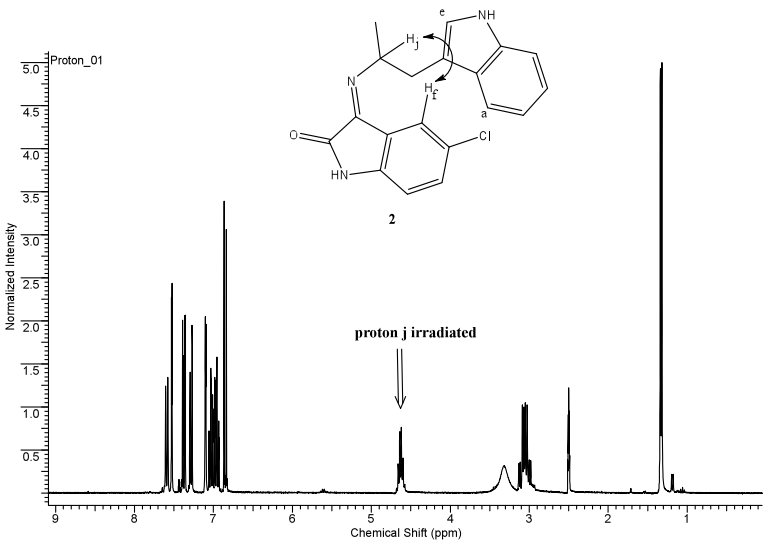
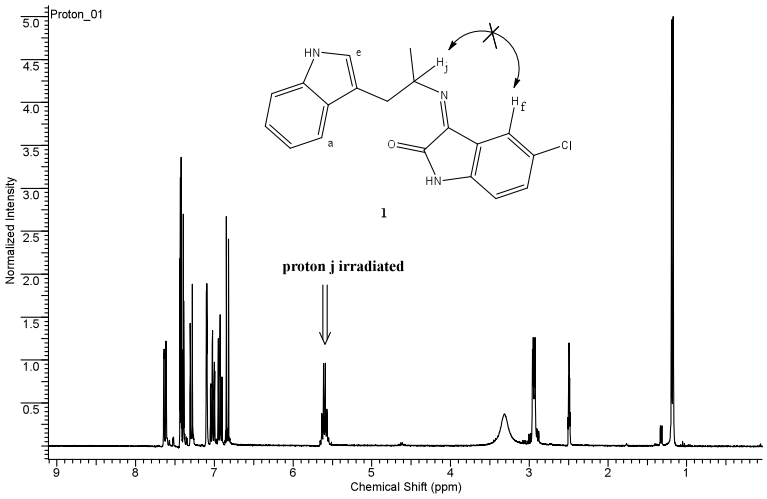


Figure 16 Structures of isomers *E* and *Z*

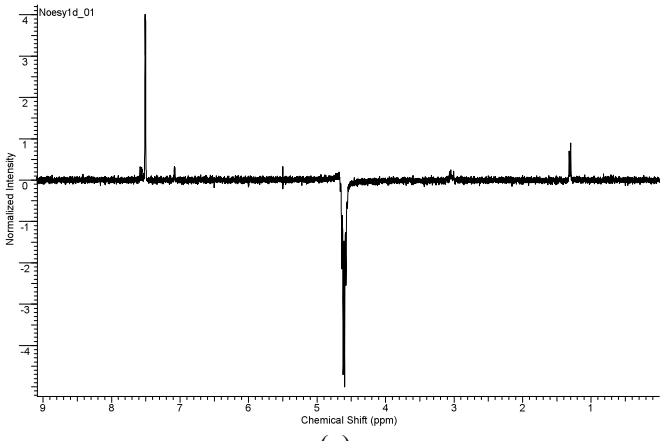
Figure 17a shows the ^{1}H NMR spectrum of imine **2**. Proton j exists as a multiplet with a chemical shift of 4.57-4.69 ppm and proton f exists as a doublet at 7.53 ppm. In contrast, proton j exists as a multiplet with a chemical shift of 5.56-5.66 ppm and proton f exists as a doublet at 7.40 ppm (Figure 17b) for imine **1**. When proton j of imine **2** was irradiated, an NOE correlation was observed with protons f, e and a (Figure 17c). Further irradiation of proton f also showed a correlation with proton j. This strong NOE correlation between protons f and j is a direct proof that imine **2** is the *E* isomer. On the other hand, imine **1** was confirmed to be the *Z* isomer as NOE correlation was only observed between protons j, e and a when proton j was irradiated.



(a)



(b)



(c)

Figure 17 NMR spectra of imines 1 (b) and 2 (a & c) in DMSO-d_6

Besides differences in NOE correlation, imines **1** and **2** differ in other physical properties too. Imine **1** is a light yellow solid with a melting point range of 181.3-182.0ºC while imine **2** is a bright yellow solid with a melting point range of 168.0-169.2ºC. In addition, infrared (IR) spectroscopic analysis of the imines showed two distinct wavelengths for the imine bond. A summary of the differences between the *E* and *Z* isomers, which would allow their differentiation, is listed in Table 1.

	Z-isomer (Imine 1)	**E-isomer (Imine 2)**
Physical appearance	Light yellow solid	Bright yellow solid
Concentration of reaction mixture at which it precipitated out	0.95 M	2.87 M
Total time of reaction (time at which the precipitate was first observed during the reaction)	1 hour 30 minutes (40 minutes)	1 hour (5 minutes)
NOE Correlation (Figure 15)	Correlation between protons e, a and j only	Correlation between protons f, e, a and j
Isomer ratio (Z:E)	20:1	1:23
Melting Point	181.3-182.0ºC	168.0-169.2ºC
Imine Bond (C=N) IR	1707 cm^{-1}	1728 cm^{-1}

Table 1 Unique characteristics of *E* and *Z* isomers

2.1.2.Stability of imines and observation of a thermodynamic mixture

Imines **1** and **2** are stable in solid form but decompose when subjected to standard HPLC conditions described under the experimental sections. When both imines were dissolved in dimethylsulfoxide (DMSO) and left to stand in solution at ambient temperature for 24 hours, a 3:1 thermodynamic mixture was observed (Figure 18). No further isomerization was detected thereafter. It is proposed that imines **1** and **2** are both kinetic products obtained through precipitation from the reaction mixture. When more time was given for inter-conversion of the isomers to take place in solution, both equilibrated thermodynamically to a common point.

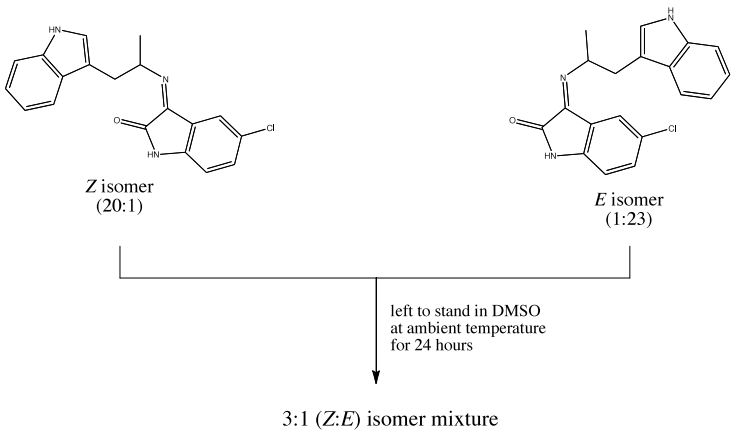


Figure 18 Equilibration of Z and E isomers to a common thermodynamic point

From the above finding, it was inferred that the *Z* isomer is more stable than the *E* isomer. Conformational effects could possibly explain the observed stabilities with the isomers. The *E* isomer adopts a more sterically hindered configuration (Figure 19) than the *Z* isomer and its formation is therefore less energetically favored as seen from the ratio of the thermodynamic mixture. It was further hypothesized that if a bulky group was substituted at the 4-position, a larger amount of steric hindrance observed in the *E* isomer would cause the formation of the *Z* isomer to be even more favorable.

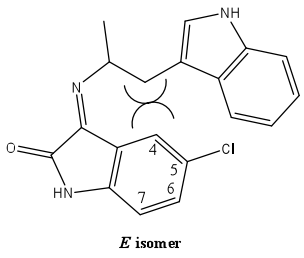
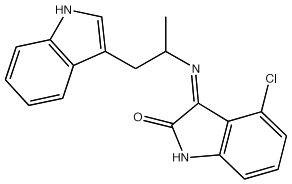


Figure 19 Steric hindrance observed in the *E* configuration

2.2. Investigation of imine formed between methyl tryptamine and 4-chloroisatin

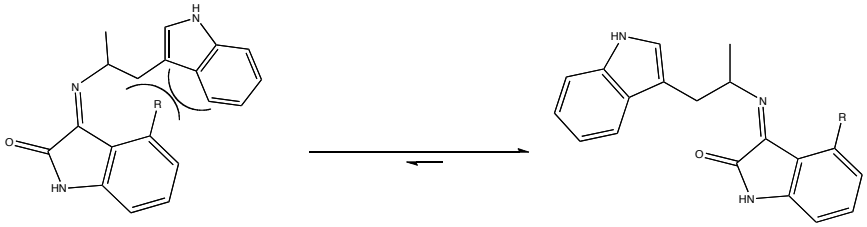
2.2.1. Synthesis and stability of imine 3

Imine **3** (Figure 20) was formed exclusively as its *Z* isomer in the reaction between methyl tryptamine and 4-chloroisatin under the conditions of 80ºC ethanol at 0.95M concentration. This result was in agreement with our previous prediction that a group bulkier than hydrogen at the 4-position of the isatin ring would have steric interactions with the indole ring and reduce the chance of the *E* configuration from being adopted (Figure 21). In the case of 4-chloro, the formation of the *E* isomer was prevented.



3

Figure 20 Structure of imine 3



***E* isomer** ***Z* isomer**

Figure 21 Steric effects contributed by a bulky R group at the 4-position would reduce the chance of the formation of the *E* isomer

Unlike imines **1** and **2**, imine **3** does not equilibrate when left to stand in DMSO solution for 24 hours. The *Z* isomer remained in solution without converting to the *E* isomer. This observation is another indication of the steric effects discussed above.

2.3. Investigation of imines formed between methyl tryptamine and 4-substituted isatins

2.3.1. Synthesis of imines 4-6

After steric hindrance at the 4-position of the isatin ring had been shown to affect the stereochemistry of imine **3**, investigation of electronic effects at the same position on the stereochemistry of the imine intermediates were carried out. Imines **4** and **5** (Figure 22) with substituents of different electronegativities, namely fluoride and methoxy, were synthesized. Imine **6** (Figure 22), formed from methyl tryptamine and isatin, served as a reference point.

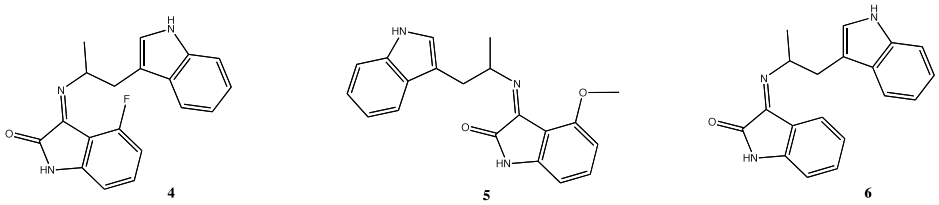


Figure 22 Structures of imines 4-6

During the initial synthesis of imine **4** at 80ºC ethanol with a reaction concentration of 0.95M, the cyclized product, as illustrated in Figure 23, was obtained instead. The ease of cyclization of imine **4** could be due to the electron withdrawing effect of fluorine, which might cause the imine bond to be more electrophilic. As the electrophilicity of the imine bond is the driving force for cyclization (17), this in turn might have led to a greater probability of attack at the imine bond (Figure 24).

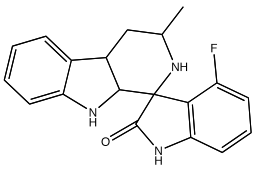


Figure 23 Structure of cyclized product obtained from the initial synthesis of imine 4

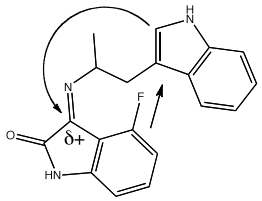


Figure 24 Electron-withdrawing effect of fluorine makes the partial positive center more positive

To prevent the cyclization of the imine **4** from taking place, the temperature of the reaction was reduced to 50ºC and the reaction concentration was increased to 2.87M. With this set of reaction conditions, imine **4** was obtained as an orange gum with an isomer (*Z*:*E*) ratio mixture of 1:3.

Both imines **5** and **6** were prepared by condensation of methyl tryptamine with 4-methoxyisatin and isatin respectively under the conditions of 80ºC ethanol at 0.95M concentration. Imine **5** was obtained as a red brown gum with an isomer ratio of 4:1 and imine **6** was isolated as a brown gum with an isomer ratio of 1:3. No sign of cyclization was observed during the synthesis of these imines.

2.3.2. Initial ratios and stability of imines 4-6

Imines **4-6** were dissolved in DMSO and ^{1}H NMR was used to determine the initial ratios of these isomer mixtures. To observe the stability of these imines, the mixtures were left in solution at room temperature and the isomer ratios were re-determined after 24 hours (Table 2).

Imine	**Isomer ratio (*Z*:*E*)**	**Isomer ratio when left to stand for 24 hours in DMSO (*Z*:*E*)**
4	1:3	2:1
5	4:1	9:1
6	1:3	2:1

Table 2 Comparison of isomer ratios of imines 4-6

The results demonstrated that electronic effects at the 4-position do not influence the configuration of the imine. Instead, steric effects play a more important role. When a bulky group, such as methoxy, was introduced, the initial ratio of imine **5** was in favor of *Z*. Smaller substituents, such as fluoride and hydrogen, provided initial ratios in favor of *E* as seen in imine **4** and **6**. These ratios reflected the isomer mixture at the point of termination of the reactions and showed the favorable formation of the *Z* configuration in the presence of steric bulk at the 4-position. However, once the imines were allowed to equilibrate at room temperature, all the ratios shifted to favor the *Z* isomer.

When chloride was introduced at the 4-position as mentioned previously in section 2.2.1, the *Z* isomer was formed exclusively in imine **3**. Both chloride and methoxy give similar steric effects but in terms of electronic effects, chloride is electron-withdrawing while methoxy is electron-donating. Despite the differences in electronic effects, both substituents produce imines that contain the *Z* isomer predominantly and this further supports our previous argument that a bulky group at the 4-position would lead to the formation of the *Z* configuration, which is energetically favored as it has less steric interactions than the *E* configuration.

2.4. Cyclization of the imine intermediates

2.4.1. Conditions of the cyclizations

The next stage of this study involved the acid-catalyzed cyclizations (Figure 25) of imine intermediates **1**-**3** at -78ºC, room temperature and 110ºC for 1 hour, 25 minutes and 10 minutes respectively. 10 equivalents of hydrochloric acid were used and ethyl acetate was used as the solvent. Ethyl acetate was chosen because it could dissolve the imine intermediate and previous work in our laboratory had shown that ethyl acetate gave similar diastereoselectivity as ethanol, which was the solvent used in the original synthesis of NITD20. All reactions were carried out in both anhydrous and non-anhydrous conditions.

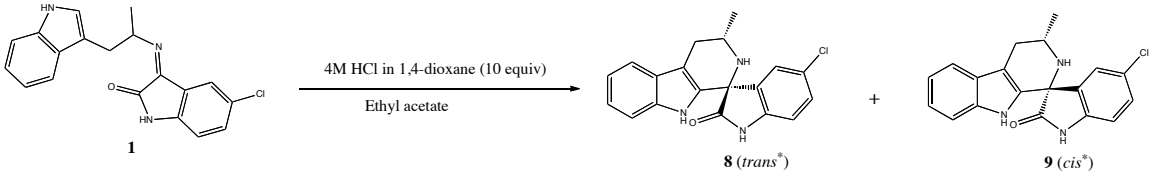


Figure 25 Acid-catalyzed cyclization of imine 1 (* all possible structures for *trans* and *cis* products were shown in Figure 11)

The influence of the reaction temperature was studied and kinetic and thermodynamic products were obtained. By conducting the reaction in both anhydrous and non-anhydrous conditions, the influence of traces of water on the stereochemical outcome of the cyclization could also be observed.

2.4.2. Diastereoselectivities of the cyclizations of imines 1 and 2

Imine **1** was cyclized as illustrated in Figure 25 and the results are summarized in Table 3. Cyclization of imine **1** always gave the *trans* product **8** (Figure 25) as the major product with high diastereoselectivity regardless of the temperature or if traces of water were excluded or not. Under non-anhydrous conditions, the diastereomeric ratio was highest at -78ºC and decreased as the temperature of the reaction increased. When anhydrous conditions were employed instead, the diastereomeric ratios obtained at -78ºC and room temperature were similar to those obtained under the non-anhydrous conditions, showing that anhydrous conditions had no influence on the stereochemical outcome of the reaction at these temperatures. However, at 110ºC, the diastereomer ratio obtained under anhydrous conditions was about twice as high as that obtained under non-anhydrous conditions.

	Diastereomer ratio (*trans*:*cis*)	
Temperature	**Non-anhydrous ethyl acetate**	**Anhydrous ethyl acetate**
-78ºC	18:1	19:1
Room temperature	12:1	11:1
110ºC*	10:1	21:1

Table 3 Diastereomer ratio of cyclized products of imine 1 (*reaction carried out in a sealed tube)

Imine **2** was cyclized under the same conditions as imine **1** (Figure 26) and the results are shown in Table 4. At -78ºC, cyclization of imine **2** afforded the *cis* product **9** as the major product with high diastereoselectivity. As the temperature of the reaction was increased, the diastereomeric ratio was reversed and the *trans* product **8** was formed as the major product at 110ºC. These trends were observed for both anhydrous and non-anhydrous conditions. Similar to imine **1**, the diastereomer ratio obtained under anhydrous conditions was about twice as high as that achieved under non-anhydrous conditions at 110ºC.

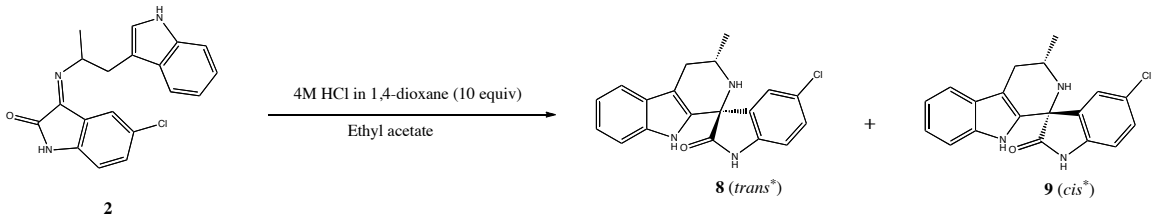


Figure 26 Acid-catalyzed cyclization of imine 2 (* all possible structures for *trans* and *cis* products were shown in Figure 11)

	Diastereomer ratio (*trans*:*cis*)	
Temperature	**Non-anhydrous ethyl acetate**	**Anhydrous ethyl acetate**
-78ºC	1:20	1:22
Room temperature	1:2	1:1
110ºC*	7:1	15:1

Table 4 Diastereomer ratio of cyclized products of imine 2 (*reaction carried out in a sealed tube)

The results obtained from the cyclizations of imines **1** and **2** suggest that at -78ºC, the configuration of the imine intermediates dictates the stereochemistry of the cyclized products. The *E* isomer (imine **2**) will cyclize to give the *cis* product **9** while the *Z* isomer (imine **1**) will cyclize to give the *trans* product **8** at -78ºC. As the temperature was increased, such control was lost. At 110ºC, the imine configuration has no influence on the stereochemical outcome of the

reaction as both isomers cyclized to give the *trans* product. The increase in diastereomeric ratio at 110ºC under anhydrous conditions was reproducible but no explanation could be offered at this point of time. An isotopic labeling experiment using deuterated water could be carried out to examine the role of water in the reaction and the results obtained might help to explain such an observation.

To probe the cyclization conditions of the actual Pictet-Spengler reaction of NITD20, the 3:1 (*Z*:*E*) thermodynamic mixture was cyclized. Equilibrating either of the imines preformed the 3:1 mixture of imine **1** and **2**. Cyclizations were carried out at the three different temperatures and under non-anhydrous conditions (Figure 27). Results of these reactions are summarized in Table 5.

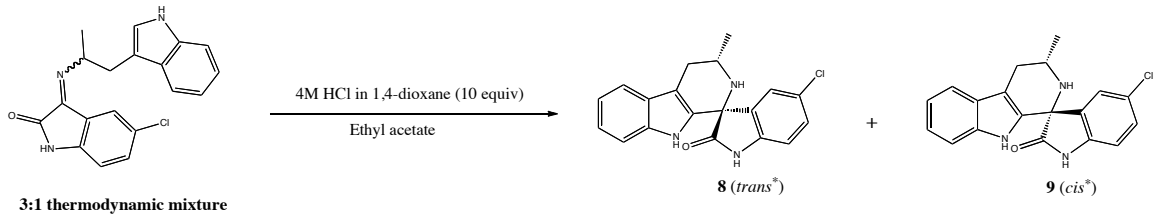


Figure 27 Acid-catalyzed cyclization of thermodynamic mixture (* all possible structures for *trans* and *cis* products were shown in Figure 11)

	Diastereomer ratio (*trans*:*cis*)
Temperature	**Non-anhydrous ethyl acetate**
-78ºC	1:1
Room temperature	3:1
110ºC*	11:1

Table 5 Diastereomer ratio of cyclized products of thermodynamic mixture (*reaction carried out in a sealed tube)

Similar to the cyclizations of imines **1** and **2** separately, the formation of the *trans* product **8** was observed for the cyclizations of the thermodynamic mixture at high temperatures. Regardless of the ratios (*Z*:*E*) of the imines (20:1 for imine **1**; 1:23 for imine **2**; 3:1 for the

thermodynamic mixture) cyclized under non-anhydrous conditions at 110ºC, similar diastereomeric ratios (*trans*:*cis*) of the cyclized products (10:1 for the product of imine **1**; 7:1 for the product of imine **2**; 11:1 for the product of thermodynamic mixture) were achieved. These findings suggest that under thermodynamic conditions, the configuration of imine is not the source of stereochemical control but other intermediates of the reaction are determining the stereochemical outcome.

2.4.3. Heating of cyclized products of imines 1 and 2

Previously, in section 1.3, the isomerization of the tetrahydro-β-carboline compounds was illustrated in Figure 7. This mechanism was suggested to occur under acidic conditions and high temperatures and provide thermodynamic mixtures of the tetrahydro-β-carboline compounds (17). In order to determine whether such a mechanism was providing the stereochemical control for the cyclized products in this study, *trans* product **8** and *cis* product **9** were heated separately at 110ºC in a sealed tube for 23 hours. 10 equivalents of hydrochloric acid were utilized and ethyl acetate was used as the solvent.

	***trans* product 8**	***cis* product 9**
Diastereomer ratio of product before heating (*trans*:*cis*)	17:1	1:12
Diastereomer ratio of product after heating for 23 hours (*trans*:*cis*)	14:1	1:4

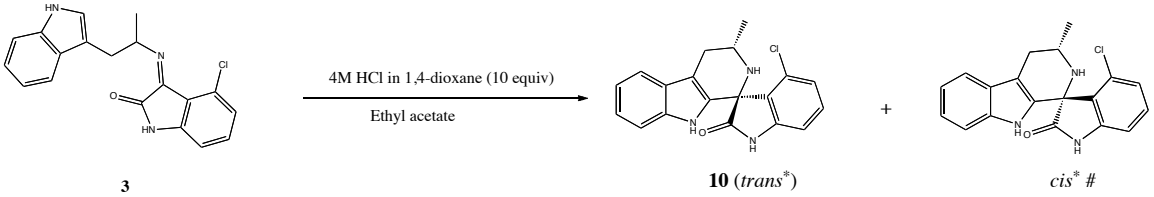
Table 6 Diastereomer ratios of *trans* and *cis* products before and after heating

The results in Table 6 showed that isomerization of the cyclized products did not contribute significantly to the diastereoselectivity of the cyclization of the imine intermediates. A decrease in diastereomeric ratio for the *cis* product **9** was observed but even with 23 hours of heating, it was not enough to reverse the ratio to give the *trans* product **8**. On the other hand,

when imine **2** was cyclized at 110ºC as mentioned in section 2.4.2, the diastereomeric ratio was reversed and the *trans* product **8** was generated after an hour of heating. In the case of this study, the isomerization of the cyclized products is probably too slow to have an effect on the stereochemistry of the products.

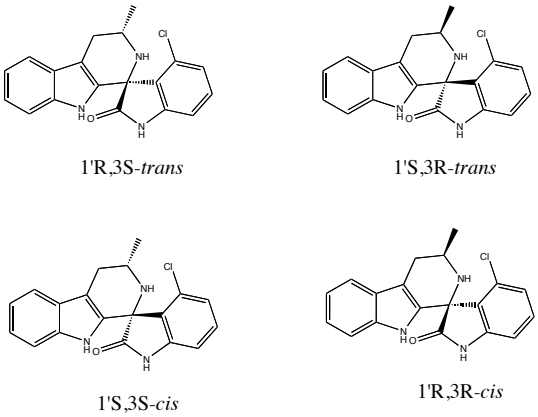
2.4.4. Diastereoselectivity of the cyclization of imine 3

Cyclization of imine **3** was performed under the same conditions as imines **1** and **2** (Figure 28). The results are summarized in Table 7.



3 **10** (*trans**) *cis** #

Figure 28 Acid-catalyzed cyclization of imine 3 (* all possible structures for trans and cis products were shown in Figure 29; # *cis* product was not characterized)



1'R,3S-*trans* 1'S,3R-*trans*

1'S,3S-*cis* 1'R,3R-*cis*

Figure 29 Possible structures of *trans* and *cis* products from the cyclization of imine 3

	Diastereomer ratio (*trans*:*cis*)	
Temperature	**Non-anhydrous ethyl acetate**	**Anhydrous ethyl acetate**
-78ºC	165:1	123:1
Room temperature	37:1	46:1

110ºC*	12:1	18:1

Table 7 Diastereomer ratio of cyclized products of imine 3 (*reaction carried out in a sealed tube)

Cyclization of imine **3** always gave the *trans* product **10** as the major product. The *cis* product was never obtained predominantly. At -78ºC, the *trans* product **10** was formed in major excess since the *Z* isomer was formed exclusively during the synthesis of imine **3**. As the temperature increased, the diastereomeric ratio decreased but the *trans* product remained the major product. This trend was observed for both non-anhydrous and anhydrous conditions.

3. General Discussion

Analogous to the mechanism proposed by Bailey et al. (Figure 9), the general mechanism of the Pictet-Spengler reaction between methyl tryptamine and 5-chloroisatin is shown in Figure 30. Methyl tryptamine and 5-chloroisatin react to form imine intermediate **a**, which has to form a 6-membered ring carbonium ion **c** before the final product is generated. The imine intermediate **a** could also form the 5-membered ring spiroindolenine intermediate **b** instead. However, this step of the reaction had already been proven in the literature to be fast and reversible and would not be expected to affect the stereochemistry of the final indoline-spiro-tetrahydro-β-carboline product **d**. Therefore, the configuration of the imine intermediate **a** was hypothesized to be an important factor in the formation of carbonium ion **c**, which would further determine the stereochemistry of the final product **d**.

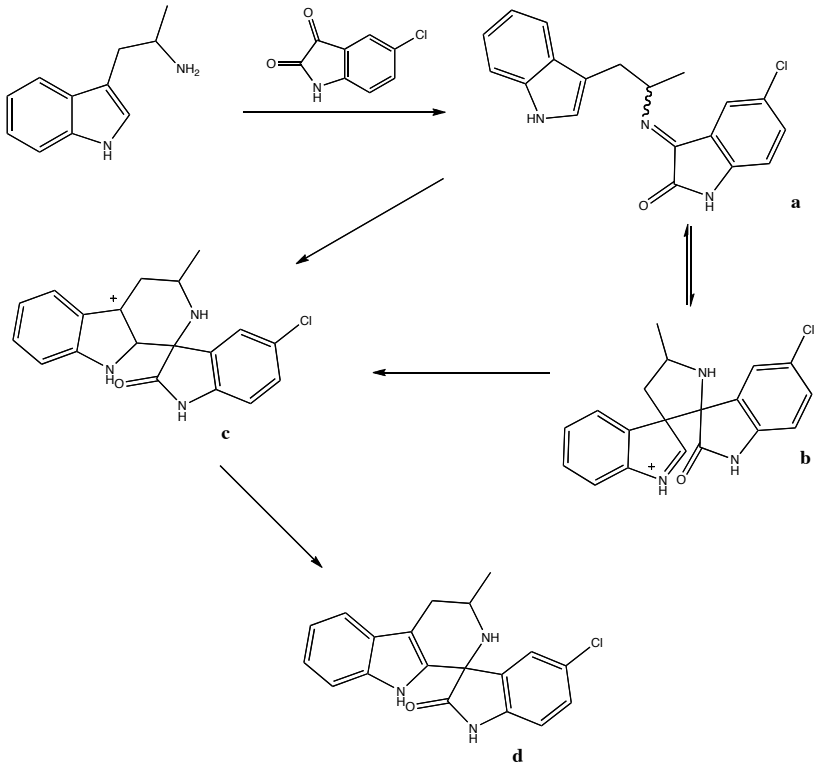


Figure 30 Proposed mechanisms for the Pictet-Spengler reaction between methyl tryptamine and 5-chloroisatin

3.1. Imine configuration as a source of stereochemical control under kinetic conditions

The results of this study support the hypothesis that the source of stereochemical control for the Pictet-Spengler reaction under kinetic conditions could come from the configuration of the imine intermediates. At -78ºC, imine **1** (*Z* isomer) and imine **2** (*E* isomer) cyclized to give *trans* product **8** and *cis* product **9** respectively. These mechanisms are presented in Figures 31 and 32.

As shown in Figure 31, a chair-like transition state is assumed to form during the cyclization of imine **1**. In this transition state, both the methyl group of methyl tryptamine and the phenyl group of the isatin ring lie in preferable pseudoequatorial positions, avoiding 1,3-diaxial strain. This would lead to the prediction that the formation of the *trans* product **8** is preferred, which is in agreement with the result obtained under kinetic control.

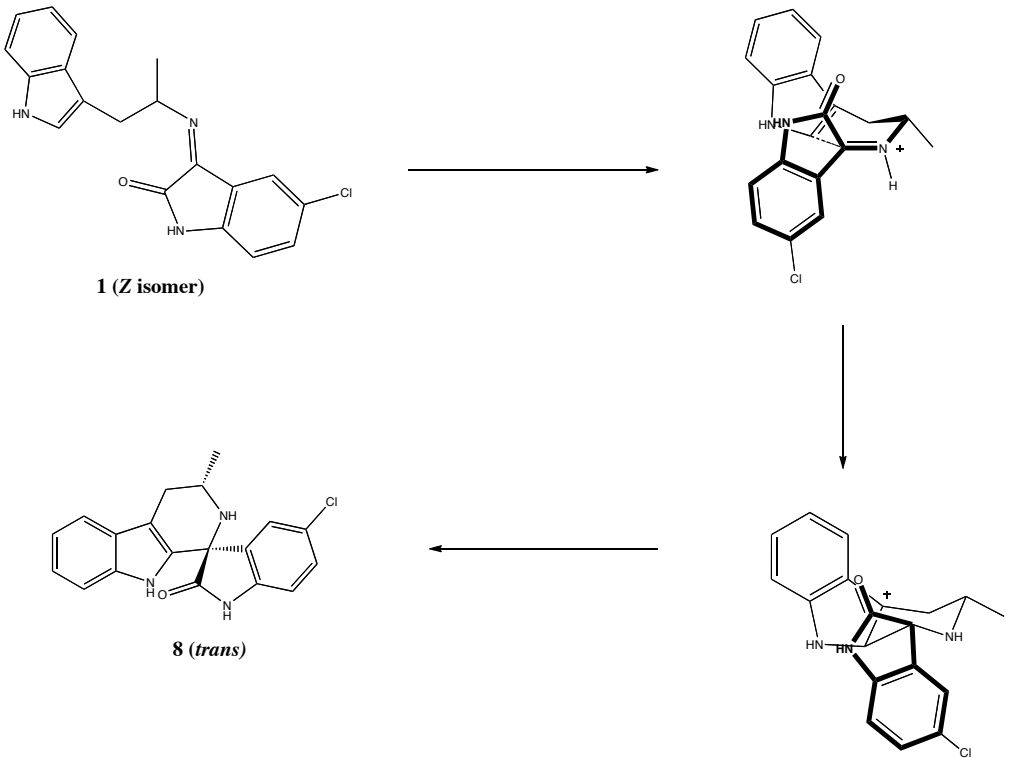


Figure 31 Proposed mechanisms for the cyclization of imine 1 at -78ºC to give the *trans* product 8

Likewise for the cyclization of imine **2**, a similar transition state (Figure 32) is formed with the methyl group in a pseudoequatorial position and the phenyl group in a pseudoaxial position. The 1,3-diaxial strain is thus avoided and this would lead to the prediction that the formation of the *cis* product **9** is preferred, which once again coincides with the experimental results obtained.

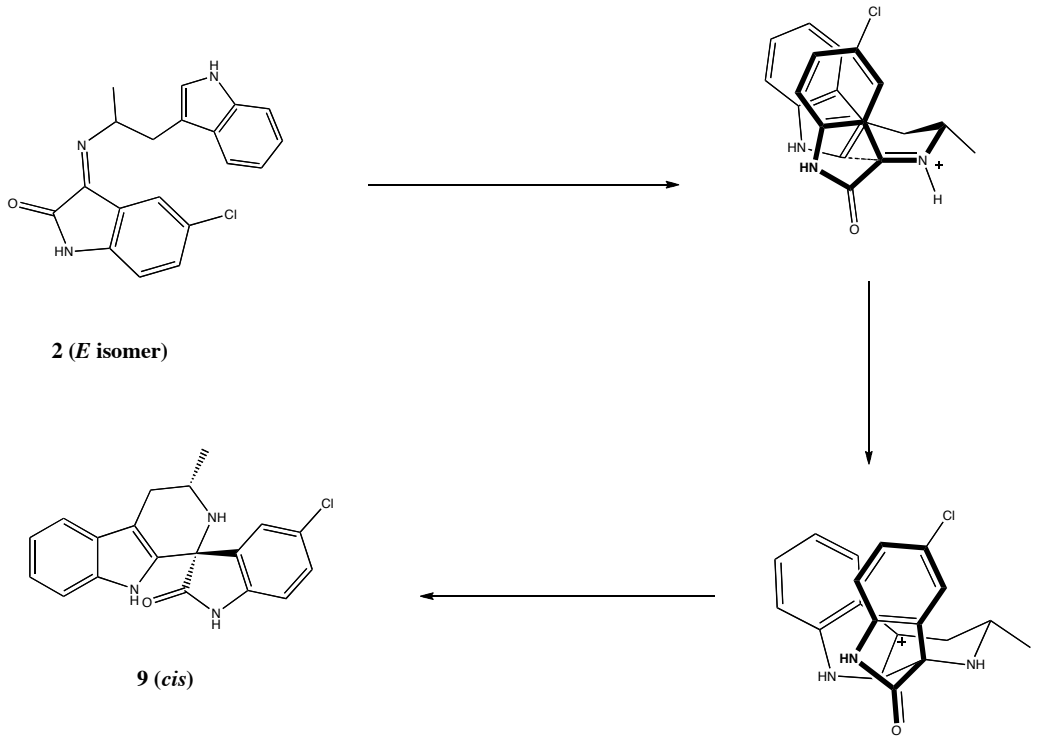


Figure 32 Proposed mechanisms for the cyclization of imine 2 at -78ºC to give the *cis* product 9

3.2. Source of stereochemical control under thermodynamic conditions

As illustrated in Figure 33, the formation of spiro intermediate **b** from imine intermediate **a** and the formation of carbonium ion **c** from both **b** and **a** are in equilibrium under thermodynamic conditions. Hence, the configuration of **a** would not be expected to affect the stereochemistry of the product. Indeed, experimental results showed that the *trans* product is formed regardless of the imine configuration at 110ºC. Furthermore, cyclization of imine **3** at

110ºC generated the *cis* product (minor product) even though imine **3** exists only in the *Z* configuration, which has been shown to preferably cyclize to the *trans* product under kinetic conditions. On the other hand, the final step in which carbonium ion **c** loses a proton to generate product **d** is irreversible and the configuration of the carbonium ion is postulated to determine the stereochemistry of the products. The carbonium ion can adopt two possible configurations (**c_1** and **c_2)**, which will each cyclize to give two different products. The phenyl group of the isatin ring in **c_1** lies in a pseudoequatorial position and will lead to the *trans* product, while the phenyl group in **c_2** lies in a pseudoaxial position, leading to the *cis* product. The pseudoequatorial position is more energetically favored and thus at 110ºC, the equilibrium lies in favor of the formation of **c_1**, leading to the generation of the *trans* product **8**. This is in agreement with our experimental results.

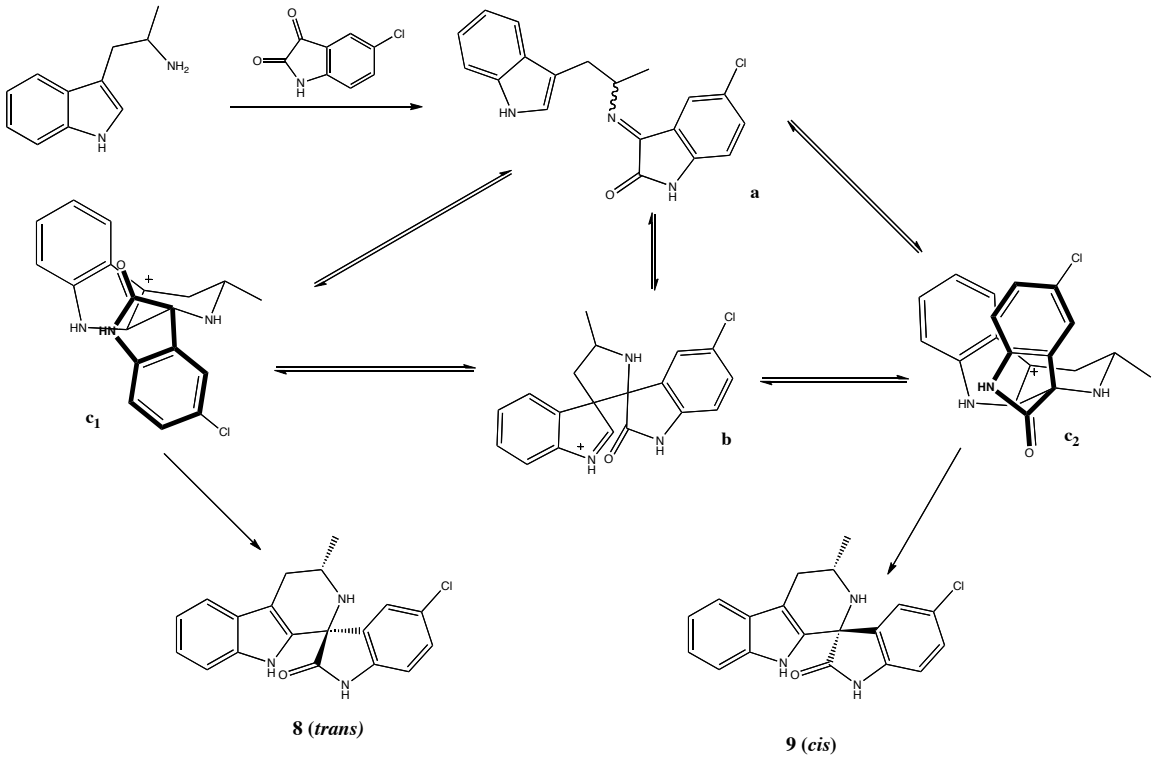


Figure 33 Proposed mechanisms for the Pictet-Spengler reaction under thermodynamic conditions

4. Conclusion and Outlook

This study investigated the importance of the configuration of the imine intermediates in the control of the stereochemistry of the products of the Pictet-Spengler reaction between methyl tryptamine and 5-chloroisatin.

The observation of a 3:1 (*Z*:*E*) thermodynamic mixture suggests that the *Z* isomer is more stable than the *E* isomer. Experimental results have also shown that under kinetic conditions, the *Z* isomer cyclized to give the *trans* product preferably while the *E* isomer cyclized to give the *cis* product preferably. Mechanisms for these cyclizations have been proposed. This direct correlation was lost when the cyclizations took place under thermodynamic conditions and both the *Z* and *E* isomers gave rise to the formation of the *trans* product predominantly. A hypothesis for this phenomenon is given and such results could explain the high diastereoselectivity, in favor of the *trans* product, observed with the Pictet-Spengler reaction for the synthesis of NITD20.

The synthesis and full characterization of the imine intermediates were also successfully carried out. The isolation of these imine intermediates allowed the investigation of the influence of the imine geometry on the diastereoselectivity of the reaction.

In conclusion, the synthesis of indoline-spiro-tetrahydro-β-carboline product through an imine intermediate has been achieved and control of the diastereoselectivity of the products has been demonstrated. This led to a better understanding of the importance of the imine intermediates in the Pictet-Spengler reaction.

5. Experimental Sections

5.1. General Methods

Materials and reagents were of the highest commercially available grade and used without further purification. Methyl tryptamine and 4-chloroisatin were obtained from the chemical archive of NITD.

Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F254 (2.5 × 7.5 cm) using UV light (λ = 254nm) for visualization. TLC data are given as the Rf value with the corresponding eluent system specified in brackets.

1H NMR and 13C NMR spectra were determined on a Varian 300 Mercury spectrometer. A relaxation delay of 2 sec and 32 repetitions were performed to obtain the 1H NMR spectra while a relaxation delay of 1 sec and 1000 repetitions were performed to obtain the 13C NMR spectra. Chemical shifts (δ) are expressed in ppm. Splitting patterns are described as singlet (s), broad singlet (br.s.), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet (t), triplet of doublet (td), quartet (q) or multiplet (m).

Infrared (IR) spectroscopic analyses were performed with an FT/IR-4100typeA machine.

Melting point determinations were made on the Buchi B-540 melting point apparatus.

LC-MS analyses were performed with an Agilent LC1100 coupled with Applied Biosystems API2000, using the following conditions: monolithic-C18, 50 × 4.6 mm column; mobile system of acetonitrile/water with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 4 mins; UV 254 and 214 nm; and flow rate of 3 mL/min. High resolution MS was done at the Department of Chemistry, National University of Singapore.

HPLC ratio determinations were made on an Agilent LC1100 HPLC, using the following conditions: SymmetryShield RP-18 3.5µm, 150 × 4.6 mm column; mobile system of acetonitrile/ water with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 20 mins; UV 254 and 214 nm; and flow rate of 0.8 mL/min.

Flash chromatography was performed using a Teledyne ISCO CombiFlash® system using RediSep® Rf disposable Flash columns. Column chromatography was done on manually packed column of Merck silica gel 60 (0.040-0.063 mm).

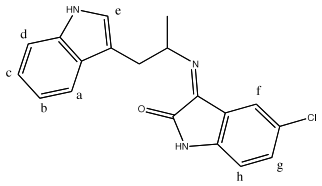
5.2. General Procedures

5.2.1. General procedure for cyclization of imines at different temperatures

The imine (1.0 eq) was suspended in ethyl acetate to give a yellow suspension. 4M hydrochloric acid (10.0 eq) in 1,4-dioxane was added to the suspension at -78ºC, room temperature or 110ºC and the reaction mixture was stirred for 1 hour, 25 minutes or 10 minutes respectively. 1M sodium hydroxide (3 mL) was added to quench the reaction, which was further extracted with ethyl acetate (2 x 8 mL). Purification of the crude mixture by column chromatography yielded the cyclized product.

5.3. Synthesis of the imine intermediates

(*Z*)-3-[(2-1H-indole-4-yl)isopropylimino]-5-chloroindolin-2-one (1)



Methyl tryptamine (100.0 mg, 0.57 mmol) and 5-chloroisatin (104.2 mg, 0.57 mmol) were dissolved in dry ethanol (0.6 mL). The resulting clear orange red solution was stirred and heated at 80ºC for 1 hour 30 minutes. A yellow precipitate was observed after 40 minutes of stirring. After completion of the reaction, the precipitate was collected via filtration, washed with ethanol and dried under vacuum. The title compound was isolated as a light yellow powder (113.0 mg, 0.33 mmol, 58% yield).

HRMS: m/z: [M+H]+ 338; [M+Na]+ 360; [M-H]- 336.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.19 (d, J = 6.15 Hz, 3H, CH_3), 2.88-3.01 (m, 2H, CH_2), 5.56-5.66 (m, 1H, CH), 6.84 (dd, J = 8.21, 0.59 Hz, Ar-H-h), 6.93 (ddd, J = 7.99, 6.96, 1.17 Hz,

1H, Ar-H-b), 7.03 (td, *J* = 7.47, 1.17 Hz, 1H, Ar-H-c), 7.10 (d, *J* = 2.34 Hz, 1H, Ar-H-e), 7.30 (d, *J* = 7.91 Hz, 1H, Ar-H-d), 7.40 (d, *J* = 2.05 Hz, 1H, Ar-H-f), 7.41 - 7.46 (m, 1H, Ar-H-g), 7.63 (d, *J* = 7.62 Hz, 1H, Ar-H-a) 10.76 (s, 1H, NH) 10.98 (br.s., 1H, NH).

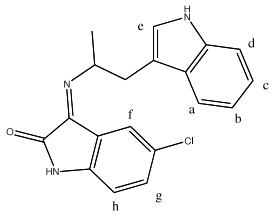
^{13}C NMR (75 MHz, DMSO-d_6): d = 21.3, 33.8, 56.2, 110.5, 110.6, 112.9, 113.0, 118.9, 119.0, 122.1, 122.2, 123.4, 127.5, 128.3, 131.8, 135.7, 140.3, 150.1, 160.2.

NOESY-1D (300 MHz, DMSO-d_6): No correlation observed between protons j, f, e and a.

Melting point: 181.3-182.0ºC.

Imine bond (C=N) IR: 1707 cm^{-1}.

(*E*)-3-[(2-1H-indole-4-yl)isopropylimino]-5-chloroindolin-2-one (2)



Methyl tryptamine (100.0 mg, 0.57 mmol) and 5-chloroisatin (104.2 mg, 0.57 mmol) were dissolved in dry ethanol (0.2 mL). The resulting clear orange red solution was stirred and heated at 80ºC for 1 hour. A yellow precipitate was observed after 5 minutes of stirring. After completion of the reaction, the precipitate was collected via filtration, washed with ethanol and dried under vacuum. The title compound was isolated as a bright yellow powder (113.0 mg, 0.33 mmol, 58% yield).

HRMS: m/z: [M+H]+ 338; [M+Na]+ 360; [M-H]- 336.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.33 (d, *J* = 6.15 Hz, 3H, CH_3), 2.97-3.14 (m, 2H, CH_2), 4.57-4.69 (m, 1H, CH), 6.85 (d, *J* = 8.50 Hz, 1H, Ar-H-h), 6.96 (ddd, *J* = 7.91, 6.74, 1.17 Hz, 1H, Ar-H-b), 7.03 (td, *J* = 7.55, 1.32 Hz, 1H, Ar-H-c), 7.10 (d, *J* = 2.34 Hz, 1H, Ar-H-e), 7.28 (ddd, *J* = 7.77, 1.03, 0.88 Hz, 1H, Ar-H-d), 7.37 (dd, *J* = 8.50, 2.05 Hz, 1H, Ar-H-g), 7.53 (d, *J* = 2.34 Hz, 1H, f), 7.59 (d, *J* = 7.62 Hz, 1H, Ar-H-a), 10.77 (s, 1H, NH) 10.93 (br.s., 1H, NH).

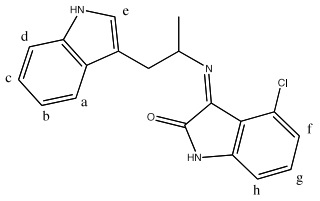
^{13}C NMR (75 MHz, DMSO-d_6): d = 20.6, 33.6, 58.9, 110.5, 111.8 112.4. 112.9, 117.2, 118.3, 119.2, 122.2, 126.0, 127.3,127.7, 131.9, 135.6, 142.3, 151.0, 158.2.

NOESY-1D (300 MHz, DMSO-d_6): Correlation observed between protons j, f, e and a.

Melting point: 168.0-169.2ºC.

Imine bond (C=N) IR: 1728 cm^{-1}.

(*Z*)-3-[(2-1H-indole-4-yl)isopropylimino]-4-chloroindolin-2-one (3)



Methyl tryptamine (100.0 mg, 0.57 mmol) and 4-chloroisatin (104.2 mg, 0.57 mmol) were dissolved in dry ethanol (0.6 mL). The resulting clear orange solution was stirred and heated at 80ºC for 2 hours 30 minutes. After completion of the reaction, the precipitate was collected via filtration, washed with ethanol and dried under vacuum. The title compound was isolated as a yellow powder (107.3 mg, 0.32 mmol, 55% yield).

HRMS: m/z: [M+H]+ 338; [M+Na]+ 360; [M-H]- 336.

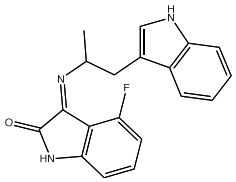
^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.20 (d, *J* = 6.15 Hz, 3H, CH_3), 2.89-3.04 (m, 2H, CH_2), 5.63-5.73 (m, 1H, CH), 6.78 (dd, *J* = 7.77, 0.73 Hz, 1H, Ar-H-h), 6.93 (ddd, *J* = 7.99, 6.96, 1.17 Hz, 1H, Ar-H-b), 6.99-7.06 (m, 2H, Ar-H-c & d), 7.13 (d, *J* = 2.34 Hz, 1H, Ar-H-e), 7.27-7.38 (m, 2H, Ar-H-g & f), 7.66 (d, *J* = 7.62 Hz, 1H, Ar-H-a), 10.76 (s, 1H, NH) 11.05 (br.s., 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6): d = 22.4, 34.7, 56.5, 109.9, 111.9, 112.4, 118.1, 118.8, 119.5, 121.4, 124.2, 124.5, 128.2, 129.6, 134.1, 136.8, 146.4, 151.7, 159.2.

Melting point: 169.4-170.3ºC.

Imine bond (C=N) IR: 1708 cm^{-1}.

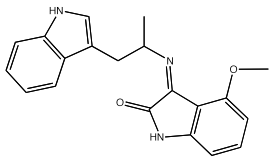
(*E*)-3-[(2-1H-indole-4-yl)isopropylimino]-4-fluoroindolin-2-one (4)



Methyl tryptamine (100.0 mg, 0.57 mmol) and 4-fluoroisatin (94.7 mg, 0.57 mmol) were dissolved in dry ethanol (0.2 mL). The resulting clear orange solution was stirred and heated at 50ºC for 2.5 hours. After completion of the reaction, the volatile solvents were removed under high vacuum. The title compound was isolated as an orange gum (91.9 mg, 0.29 mmol, 100% yield).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.31 (d, J = 6.15 Hz, 3H, CH_3), 3.09 (d, J = 6.15 Hz, 2H, CH_2), 4.55-4.67 (m, 1H, CH), 6.67 (d, J = 9.38 Hz, 1H, Ar-H), 6.93-6.99 (m, 1H, Ar-H), 7.04 (td, J = 7.47, 1.17 Hz, 1H, Ar-H), 7.14 (d, J = 2.05 Hz, 1H, Ar-H), 7.30-7.35 (m, 2H, Ar-H), 7.56-7.61 (m, 2H, Ar-H), 10.79 (br.s., 2H, NH & NH).

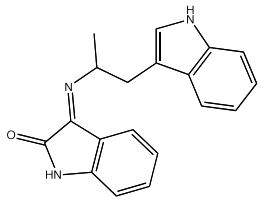
(*Z*)-3-[(2-1H-indole-4-yl)isopropylimino]-4-methoxyindolin-2-one (5)



Methyl tryptamine (100.0 mg, 0.57 mmol) and 4-methoxyisatin (101.7 mg, 0.57 mmol) were dissolved in dry ethanol (0.6 mL). The resulting clear orange solution was stirred and heated at 80ºC for 2.5 hours. After completion of the reaction, the volatile solvents were removed under high vacuum. The title compound was isolated a red brown gum (190.0 mg, 0.57 mmol, 99% yield).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.19 (d, J = 6.15 Hz, 3H, CH_3), 2.92-3.04 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 4.55-4.67 (m, 1H, CH), 6.47 (d, J = 7.74 Hz, 1H, Ar-H), 6.67 (d, J = 8.58 Hz, 1H, Ar-H), 6.95-6.99 (m, 1H, Ar-H), 7.02-7.07 (m, 1H, Ar-H), 7.30-7.34 (m, 2H, Ar-H), 7.76 (d, J = 7.86 Hz, 1H, Ar-H), 10.80 (br.s., 1H, NH), 10.83 (br.s., 1H, NH).

(*E*)-3-[(2-1H-indole-4-yl)isopropylimino]-indolin-2-one (6)

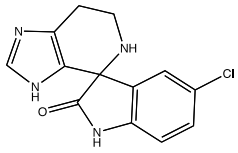


Methyl tryptamine (100.0 mg, 0.57 mmol) and isatin (84.5 mg, 0.57 mmol) were dissolved in dry ethanol (0.6 mL). The resulting clear orange solution was stirred and heated at 80ºC for 2 hours. After completion of the reaction, the volatile solvents were removed under high vacuum. The title compound was isolated as a brown gum (173.5 mg, 0.57 mmol, 100% yield).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.33 (d, J = 6.15 Hz, 3H, CH_3), 3.12 (d, J = 6.45 Hz, 2H, CH_2), 4.62-4.76 (m, 1H, CH), 6.88 (d, J = 7.91 Hz, 2H, Ar-H), 6.98 (t, J = 7.03 Hz, 1H, Ar-H), 7.06 (t, J = 7.47 Hz, 1H, Ar-H), 7.16 (d, J = 1.46 Hz, 1H, Ar-H), 7.30-7.37 (m, 2H, Ar-H), 7.59 (t, J = 7.47 Hz, 2H, Ar-H), 10.82 (br.s., 1H, NH).

5.4. Synthesis of cyclized products

5'-chloro-3,5,6,7-tetrahydrospiro[imidazo[4,5-*c*]pyridine-4,3'-indolin]-2'-one (7)



Histamine (100.0 mg, 0.90 mmol) and 5-chloroisatin (163.4 mg, 0.90 mmol) were dissolved in dry ethanol (0.9 mL). The resulting clear orange solution was stirred and heated at 80ºC for 5 hours. After completion of the reaction, the pale brown precipitate was collected via filtration, washed with ethanol and dried under vacuum. The title compound was isolated as a pale brown powder (167.8 mg, 0.61 mmol, 68% yield).

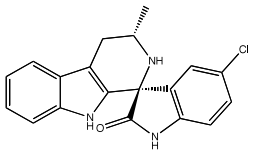
HRMS: m/z: [M+H]+ 275; [M-H]- 273.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.52-2.64 (m, 1H, CH_2), 2.66-2.78 (m, 1H, CH_2), 2.92 (br.s., 1H, NH), 2.98-3.09 (m, 1H, CH_2), 3.46-3.59 (m, 1H, CH_2), 6.83 (d, J = 8.21 Hz, 1H, Ar-H), 6.98

(d, *J* = 2.34 Hz, 1H, Ar-H), 7.22 (dd, *J* = 8.20, 2.34 Hz, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 10.30 (br.s., 1 H) 11.85 (br.s., 1 H)

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.9, 39.4, 62.6, 111.4, 125.1, 126.0 (2C), 128.8, 134.3, 136.9, 141.9 (2C), 180.0.

(1,3'*R*,3*S*)-1'*H*-spiro[(3-methyl-2,3,4,9-tetrahydro-ß-carboline)-1,3'-(5'-chloro-indol-2'-one)] (8)



(*Z*)-3-[(2-1H-indole-4-yl)isopropylimino]-5-chloroindolin-2-one **1** (20.0 mg, 0.06 mmol), suspended in ethyl acetate (0.48 mL), was cyclized according to the general procedure at -78ºC. The title compound was isolated as an off white solid (20.1 mg, 0.06 mmol, 100% yield).

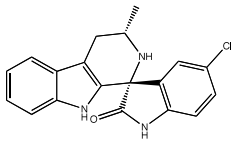
HPLC: R_t 7.60 mins (94.9%); 8.03 mins (5.1%).

LC-MS: R_t 1.52 mins; m/z (ESI): [M+H]+ 338; [M-H]- 336; R_t 1.70 mins; m/z (ESI): [M+H]+ 338; [M-H]- 336.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.18 (d, *J* = 6.15 Hz, 3H, CH_3), 2.42 (dd, *J* = 14.94, 10.55 Hz, 1H, CH_2), 2.79 (dd, *J* = 14.94, 3.52 Hz, 1H, CH_2), 3.06 (d, *J* = 5.86 Hz, 1H, NH), 3.86-4.00 (m, 1H, CH), 6.92 (d, *J* = 8.50 Hz, 1H, Ar-H), 6.95-7.01 (m, 1H, Ar-H), 7.02-7.06 (m, 2H, Ar-H), 7.17 (d, *J* = 7.91 Hz, 1H, Ar-H), 7.32 (dd, *J* = 8.94, 2.20 Hz, 1H, Ar-H), 7.44 (d, *J* = 8.21 Hz, 1H, Ar-H), 10.43 (s, 1H, NH), 10.47 (br.s., 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.4, 30.2, 45.0, 62.6, 111.7, 111.8, 111.9, 118.5, 119.1, 121.8, 125.5, 126.2, 127.1, 129.6, 131.8, 135.1, 137.1, 142.3, 179.2.

(1,3'*S*,3*S*)-1'*H*-spiro[(3-methyl-2,3,4,9-tetrahydro-ß-carboline)-1,3'-(5'-chloro-indol-2'-one)] (9)



(*E*)-3-[(2-1H-indole-4-yl)isopropylimino]-5-chloroindolin-2-one **2** (20.0 mg, 0.06 mmol), suspended in ethyl acetate (0.48 mL), was cyclized according to the general procedure at -78ºC. The title compound was isolated as an off white solid (19.0 mg, 0.06 mmol, 95% yield).

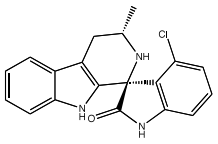
HPLC: R_t 7.53 mins (4.3%); 8.03 mins (95.7%).

LC-MS: R_t 1.63 mins; m/z (ESI): [M+H]+ 338; [M-H]- 336; R_t 1.72 mins; m/z (ESI): [M+H]+ 338; [M-H]- 336.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.23 (d, J = 6.45 Hz, 3H, CH_3), 2.32 (d, J = 7.91 Hz, 1H, NH), 2.42 (dd, J = 15.38, 10.40 Hz, 1H, CH_2), 2.91 (dd, J = 15.24, 3.81 Hz, 1H, CH_2), 3.42-3.54 (m, 1H, CH), 6.94-7.00 (m, 1H, Ar-H), 6.97 (d, J = 8.20 Hz, 1H, Ar-H), 7.03 (td, J = 7.40, 1.32 Hz, 1H, Ar-H), 7.14-7.20 (m, 2H, Ar-H), 7.30 (dd, J = 8.20, 2.05 Hz, 1H, Ar-H), 7.45 (d, J = 7.62 Hz, 1H, Ar-H), 10.60 (s, 1H, NH), 10.80 (br.s., 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.7, 30.4, 46.5, 63.6, 111.5, 111.8, 112.3, 118.6, 119.1, 122.0, 125.0, 126.4, 126.8, 129.2, 131.4, 136.6, 137.1, 141.3, 177.8.

(1,3'*R*,3*S*)-1'*H*-spiro[(3-methyl-2,3,4,9-tetrahydro-ß-carboline)-1,3'-(4'-chloro-indol-2'-one)] (10)



(*Z*)-3-[(2-1H-indole-4-yl)isopropylimino]-4-chloroindolin-2-one **3** (20.0 mg, 0.06 mmol), suspended in ethyl acetate (0.48 mL), was cyclized according to the general procedure at -78ºC. The title compound was isolated as an off-white solid (18.7 mg, 0.06 mmol, 94% yield).

HPLC: R_t 7.99 mins (0.8%); 8.72 mins (99.2%).

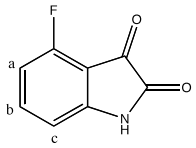
LC-MS: R_t 1.71 mins; m/z (ESI): [M+H]+ 338; [M-H]- 336.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.19 (d, J = 6.45 Hz, 3H, CH_3), 2.36 (dd, J = 14.94, 10.55 Hz, 1H, CH_2), 2.67 (d, J = 5.57 Hz, 1H, NH), 2.84 (dd, J = 14.94, 3.52 Hz, 1H, CH_2), 3.87-3.99 (m, 1H, CH), 6.85-6.94 (m, 2H, Ar-H), 6.94-7.05 (m, 2H, Ar-H), 7.17 (d, J = 7.33 Hz, 1H, Ar-H), 7.29 (t, J = 7.91 Hz, 1H, Ar-H), 7.44 (d, J = 6.74 Hz, 1H, Ar-H), 10.51 (s, 1H, NH), 10.59 (br.s., 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.5, 30.4, 45.0, 63.0, 109.3, 111.8, 112.0, 118.4, 118.9, 121.6, 123.1, 127.0, 128.9, 130.1, 131.2, 131.6, 137.1, 145.5, 178.3.

5.5. Synthesis of isatins

4-Fluoro-1H-indole-2,3-dione (11)



Chloral hydrate (1.79 g, 10.8 mmol) and sodium sulphate (10.23 g, 72.0 mmol) were dissolved in water (30 mL) and warmed up to 50ºC. 3-Fluoroaniline (0.87 mL, 9.0 mmol) was added to the reaction mixture, followed by 1M hydrochloric acid (6.1 mL). Hydroxylamine hydrochloride (2.25 g, 32.4 mmol) dissolved in water (20 mL) was subsequently added and the resulting mixture was subjected to reflux at 55ºC for 19 hours to give a cream colored precipitate. It was collected via filtration, washed with water and dried under vacuum. The dried precipitate was added in small portions to concentrated sulfuric acid (5.5 mL), which was pre-heated to 55ºC. During the addition, the temperature of the mixture was maintained between 55-65ºC and raised to 80ºC for 15 minutes after all the precipitate had been added. It was then left to cool on ice and the cooled mixture was poured onto crushed ice (27 mL) and left to stand for 1 hour. A yellow precipitate was obtained and collected via filtration. The yellow precipitate was further dissolved in 2.5M sodium hydroxide (7 mL), which was pre-heated to 60ºC, and the solution was acidified with acetic acid (10 mL) at 35ºC. This mixture was left to stand overnight to obtain a yellow

precipitate. Filtration of the mixture afforded the title compound as a yellow crystalline solid (373.3 mg, 2.26 mmol, 25% yield).

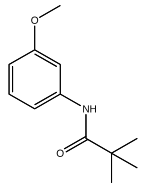
LC-MS: R_t 1.16 mins; m/z (ESI): [M-H]- 164.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.72 (dd, *J* = 9.23, 2.20 Hz, 1H, Ar-H), 6.85 (td, *J* = 9.08, 2.34 Hz, 1H, Ar-H), 7.59 (dd, *J* = 8.06, 5.71 Hz, 1H, Ar-H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 100.2 (d, *J* = 27.09 Hz), 109.4 (d, *J* = 23.77 Hz), 114.7, 127.7 (d, *J* = 11.61 Hz), 153.48 (d, *J* = 13.82 Hz), 159.7, 166.3, 169.7.

COSY (300 MHz, DMSO-d_6): Proton b shows correlation with both a and c while proton a and c show correlation with only b.

N-(3-Methoxy-phenyl)-2,2-dimethyl-propionamide (12a)



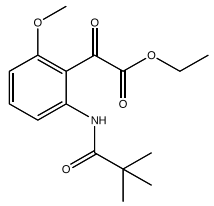
m-Anisidine (4.5 mL, 40.6 mmol) was suspended in aqueous sodium bicarbonate (20 mL) and pivaloyl chloride (5 mL, 40.6 mmol), dissolved in dichloromethane (20 mL) was added slowly to the suspension at 0ºC. The reaction was stirred at 0ºC for 1 hour 20 minutes and an off white precipitate was finally obtained. Dichloromethane (20 mL) was added to dissolve the precipitate and extract the product from the aqueous layer. The organic layer was collected, dried over sodium sulfate and evaporated to dryness. The title compound was obtained as an off white solid (6.98 g, 33.7 mmol, 83% yield).

TLC: R_f = 0.7 (hexanes:ethyl acetate 1:1).

LC-MS: R_t 2.00 mins; m/z (ESI): [M+H]+ 208.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.32 (s, 9H, $(CH_3)_3$), 3.81 (s, 3H, O-CH_3), 6.64-6.70 (m, 1H, Ar-H), 6.91-6.97 (m, 1H, Ar-H), 7.21 (t, *J* = 8.20 Hz, 1H, Ar-H), 7.33 (br.s., 1H, NH), 7.40 (t, *J* = 2.34 Hz, 1H, Ar-H).

[2-(2,2-Dimethyl-propionylamino)-6-methoxy-phenyl]-oxo-acetic acid ethyl ester (12b)



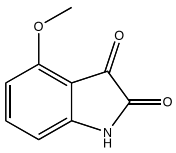
N-(3-Methoxy-phenyl)-2,2-dimethyl-propionamide **12a** (6.95 g, 33.5 mmol) was dissolved in anhydrous tetrahydrofuran (70 mL). 1.6M butyl lithium in hexanes (50.3 mL, 80.4 mmol) was added dropwise to the reaction mixture which was left to stir at 0ºC for 3 hours 50 minutes. Diethyl oxalate (5.5 mL, 40.4 mmol) was added at -78ºC and the reaction mixture was stirred for another 2 hours. The reaction was quenched with 1M hydrochloric acid (36 mL) and extracted with diethyl ether (3 x 100 mL). The organic layers were collected, dried over sodium sulfate and evaporated to dryness. The crude mixture was purified by flash chromatography (hexanes:ethyl acetate 4:1). The title compound was obtained as a pale yellow solid (8.09 g, 26.3 mmol, 79% yield).

TLC: R_f = 0.3 (hexanes: ethyl acetate 4:1).

LC-MS: R_t 2.50 mins; m/z (ESI): [M+H]+ 308; [M-H]- 306.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.21 (s, 9H, $(CH_3)_3$), 1.28 (t, *J* = 7.03 Hz, 3H, CH_3), 3.82 (s, 3H, O-CH_3), 4.30 (q, *J* = 7.03 Hz, 2H, CH_2), 6.93 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 7.62 (t, *J* = 8.35 Hz, 1H, Ar-H), 7.82 (dd, *J* = 8.35, 0.73 Hz, 1H, Ar-H), 10.61 (br.s., 1H, NH).

4-Methoxy-1H-indole-2,3-dione (12)



[2-(2,2-Dimethyl-propionylamino)-6-methoxy-phenyl]-oxo-acetic acid ethyl ester **12b** (2.15 g, 7.01 mmol) was dissolved in dimethoxyethane (30 mL). Aqueous 12N hydrochloric acid (1.75 mL, 21.0 mmol) was added and the reaction mixture was refluxed at 86ºC for 21 hours 20

minutes. The volatile solvents were removed under reduced pressure and a red precipitate was observed. Filtration of the suspension yielded the title compound was obtained as a red crystalline solid (0.88 g, 5.0 mmol, 71% yield).

LC-MS: R_t 1.01 mins; m/z (ESI): [M+H]+ 178; [M-H]- 176.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.86 (s, 3H, O-CH_3), 6.45 (d, J = 7.62 Hz, 1H, Ar-H), 6.69 (d, J = 8.50 Hz, 1H, Ar-H), 7.54 (dd, J = 8.64, 7.77 Hz, 1H, Ar-H), 11.00 (br.s., 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.5, 105.0, 106.5, 107.5, 141.4, 152.0, 158.8, 160.2, 181.2.

References

1) Warrell D.A. & Gilles H.M. *Essential Malariology; Fourth edition.* (2002).

2) Greenwood B.M, Fidock D.A., Kyle D.E., Kappe S.H., Alonso P.L., Collins F.H. & Duffy P.E. Malaria: progress, perils, and prospects for eradication. *J. Clin. Invest.* **118**: 1266-1276 (2008).

3) World Health Organization. *Africa Malaria Report 2003.* (WHO, Geneva, 2003).

4) World Health Organization. *The World Malaria Report 2008.* (WHO, Geneva, 2008).

5) United Nations Development Programme. *Human Development Report 2003. Millennium Development Goals: a Compact among Nations to End Human Poverty* (Oxford Univ. Press, Oxford, 2003).

6) Daemmrich A. & Bowden M.A. The top pharmaceuticals that changed the world. *Chem. Eng. News*. **83**: 3-12 (2005).

7) Making a difference. The World Health Report 1999. *Health Millions.* **25**: 3-5. (WHO, Geneva, 1999).

8) Mehta SR & Das S. Management of malaria: recent trends. *J. Commun. Dis.* **38**: 130-138 (2006).

9) White N. J. Antimalarial drug resistance. *J. Clin. Invest.* **113**: 1084-1092 (2004).

10) Thierry Diagana (Personal communication, 10th October, 2008).

11) Pictet, A., Spengler, T. *Ber. Dtsch. Chem. G'es.* **44**: 2030 (1911).

12) Nielsen T.E., Diness F. & Meldal M. The Pictet-Spengler reaction in solid phase combinatorial chemistry. *Curr. Opin. Drug Discov. Devel.* **6**: 801-814 (2003).

13) Bailey P.D., Hollinshead S.P., McLay N.R., Morgan K., Palmer S.J., Prince S.N., Raynolds C.D. & Wood S.D. Diastereo- and enantio-selectivity in the Pictet-Spengler reaction. *J. Chem. Soc. Perkin. Trans. 1*. **1**: 431-439 (1993).

14) Bailey P.D. Direct proof of the involvement of a spiro intermediate in the Pictet-Spengler reaction. *J. Chem. Res.* **6**: 202-203 (1987).

15) Alberch L., Bailey P.D., Clingan P.D., Mills T.J., Price R.A., Pritchard R.G. The *cis*-specific Pictet-Spengler reaction. *Eur. J. Org. Chem.* **9**: 1887-1890 (2004).

16) Ducrot P., Rabhi C., Thal C. Synthesis of tetrahydro-β-carbolines and studies of the Pictet-spengler reaction. *Tetrahedron*. **56**: 2683-2692 (2000).

17) Cox E.D. & Cook J.M. The Pictet-Spengler condensation: a new direction for an old reaction. *Chem. Rev*. **95**: 1797-1842 (1995).

18) Pogosyan S.A., Grigoryan N.P., Paronikyan R.G. Synthesis and anticonvulsant activity of dihydrochlorides of indole-3'-spiro-1-(1,2,3,4-tetrahydro)-β-carboline derivatives. *Pharm. Chem. J.* **41**: 527-528 (2007).

19) Kuo F.M., Tseng M.C., Yen Y.H. & Chu Y.H. Microwave accelerated Pictet-Spengler reactions of tryptophan and ketones directed toward the preparation of 1,1-disubstituted indole alkaloids. *Tetrahedron*. **60**: 12075-12084 (2004).

20) Abadi A.H., Abou-Seri S.M., Abdel-Rahman D.E., Klein C., Lozach O. & Meijer L. Synthesis of 3-substituted-2-oxoindole analogues and their evaluation as kinase inhibitors, anticancer and antiangiogenic agents. *Eur. J. Med. Chem.* **41**: 296-305 (2006).

VOLUME II:

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF AN INHIBITOR OF DENGUE PROLIFERATION

Table of Contents (Volume II)

Summary

From the Novartis compound archive, NITD10 was identified to have good anti-viral activity in cellular dengue assays. In order to utilize the chemical proteomics technology for the identification of the target of NITD10, an analog of NITD10 that could be immobilized to a solid matrix and yet retained its biological activity had to be designed and synthesized. A primary amino group was considered to be the ideal functional group suitable for covalent coupling to the solid matrix. The structure-activity-relationship (SAR) between NITD10 and its target was first explored so that a suitable site for coupling of the immobilizable compound to the solid support could be located. When a potential site to be immobilized was identified in an analog, another new analog with an acetylated primary amino group at that position would be synthesized and screened for activity. This analog would be submitted for chemical proteomics analysis if it showed good *in-vitro* anti-viral activity. Unfortunately the synthesis efforts were unable to identify a site where an acetylated primary amino group could be incorporated into the scaffold without affecting the activity of the compound. The synthetic studies and the SAR of the compound class will be presented instead.

List of Tables

List of Figures

1. Introduction

1.1. Dengue and the need for a cure

Dengue fever is currently endemic in more than one hundred countries in Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. Among these tropical and sub-tropical regions, South-East Asia and the Western Pacific are most seriously affected (1). The World Health Organization (WHO) estimated that to date 2.5 billion people, two fifths of the world's population, are at risk of dengue infection. Due to reduced mosquito control efforts, many epidemics have occurred over the past few decades (2). Annually, there are about 50 million reported cases of dengue worldwide (3).

The dengue virus belongs to a large family of viral pathogens called *Flaviviradae* and has four antigenically distinct serotypes. Infection with one of these serotypes provides immunity to only that serotype for life. The virus is transmitted to humans by the *Aedes* mosquitoes, mainly *Aedes aegypti* (4). After a viral incubation period of about eight to ten days, the mosquito will be able to transmit the virus for the rest of its life (3).

Symptoms of dengue fever include headache, rash, bone and muscular pains and leucopenia (3). Dengue hemorrhagic fever (DHF) is a more serious complication of dengue fever. It can occur during the first infection but is more commonly due to sequential infections by different serotypes of the dengue virus where antibody-dependent enhancement (5) of the infection could take place. DHF is classified by four major clinical symptoms, namely high fever, hemorrhagic tendency, thrombocytopenia and plasma leakage (6). There are approximately 500,000 cases of DHF per year and a large proportion of these cases affect children. Given the severity of its clinical manifestations, DHF has become a leading cause of hospitalization and death among children in some Asian countries (3). Studies, using the non-monetary composite index called 'disability-adjusted life years' (DALYs) to measure the total impact of both morbidity and mortality of dengue fever and DHF, have highlighted that in the developing world, dengue fever and DHF have a total socioeconomic impact of the same order of magnitude as many of the major infectious diseases, such as malaria, tuberculosis

and hepatitis (7). However, dengue has received less attention and funding from international funding agencies as compared to those major infectious diseases (7).

Currently, there is no approved vaccine or effective anti-viral drug to cure dengue. Patients infected with dengue are treated based upon their symptoms. Therefore, it is extremely urgent for more efforts and funding to be channeled into the development of a vaccine, which can target all four serotypes of the virus, or an anti-viral drug, which can inhibit the proliferation of the dengue virus.

1.2. The use of chemical proteomics in dengue drug discovery campaigns

Target finding is usually the first step in the drug discovery process of any disease area. Likewise for dengue, identification of a druggable target is important for subsequent lead optimization work. Potential targets for dengue include essential viral enzymes associated with the life cycle of dengue and host targets that interact with the virus during its replication in the host (8, 9). Many methods can be employed to find these targets. siRNA (small interfering ribonucleic acid) (10), gene knock out (10) and Cellzome's chemical proteomics technology (11) are a few examples.

The approach presented in this study is the Cellzome's chemical proteomics technology (11). In this technology, the active compound is attached to a solid support. Since most of the compounds of interest usually do not contain functional groups suitable for covalent coupling to the solid matrix, analogs (also known as immobilizable compounds) containing primary amino group, which can be coupled to the solid support via an amide bond while maintaining the intrinsic biological activity of the compound, are synthesized. An example is shown in Figure 1.

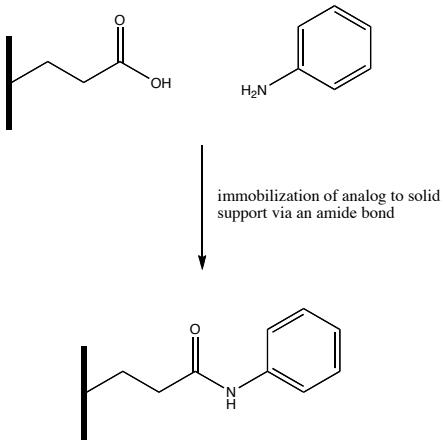


Figure 1 Immobilization of the analog to the solid support

By running cell lysates over these tethered compounds, specific protein target-compound interactions could be captured (Figure 2). The bound proteins are eluted out with an affinity gradient and digested with trypsin. The genuine protein targets are then identified by mass spectrometric analysis. Once these protein targets are validated with assays, such as site-directed mutagenesis (12), they can be the starting points for drug discovery campaigns.

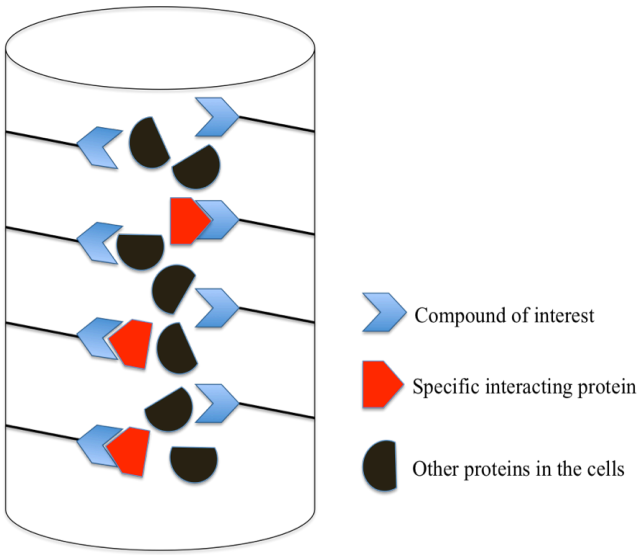


Figure 2 A simple illustration of the chemical proteomics technology

1.3. Identification of NITD10 as an inhibitor of dengue proliferation

From the Novartis compound archive, NITD10 (Figure 3) was determined to have good anti-viral activity (IC_{50} of 0.5 µM) in cellular dengue assays. However, the target of NITD10 was not known and a structure-based drug discovery program was not possible

without having a validated and druggable target. With the design and synthesis of an immobilizable compound of NITD10, the chemical proteomics technology could be utilized to identify the target of NITD10.

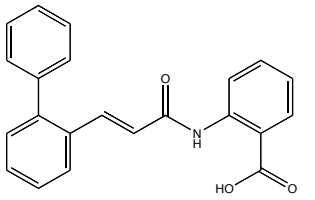


Figure 3 Struture of NITD10

To be suitable for coupling onto the solid matrix, the immobilizable compound has to contain a primary amino group and still retain an IC_{50} activity of less than 1.0μM in the cellular dengue assay. In order to aid the incorporation of the primary amino group onto an appropriate site on the scaffold of NITD10, the structure-activity relationship (SAR) of NITD10 with its target has to be established.

Herein, we report the synthesis of analogs of NITD10 in an attempt to find an immobilizable compound for target pull-down using the chemical proteomics technology. The SAR established will also be highlighted.

2. Results

Modifications were made at sites A, B and C (Figure 4) for the synthesis of analogs. The SAR of NITD10 was first explored with analogs that do not contain a primary amino group. When a potential site to be coupled was identified in an analog, a new analog **a** with a primary amino group at that position would be synthesized and tested for activity. The primary amino group would also be further acetylated to give analog **b**. This step would mimic the amide bond formation and determine whether analog **a** would lose its biological activity when tethered to the solid support. If analog **b** has an IC_{50} activity of less than 1.0μM

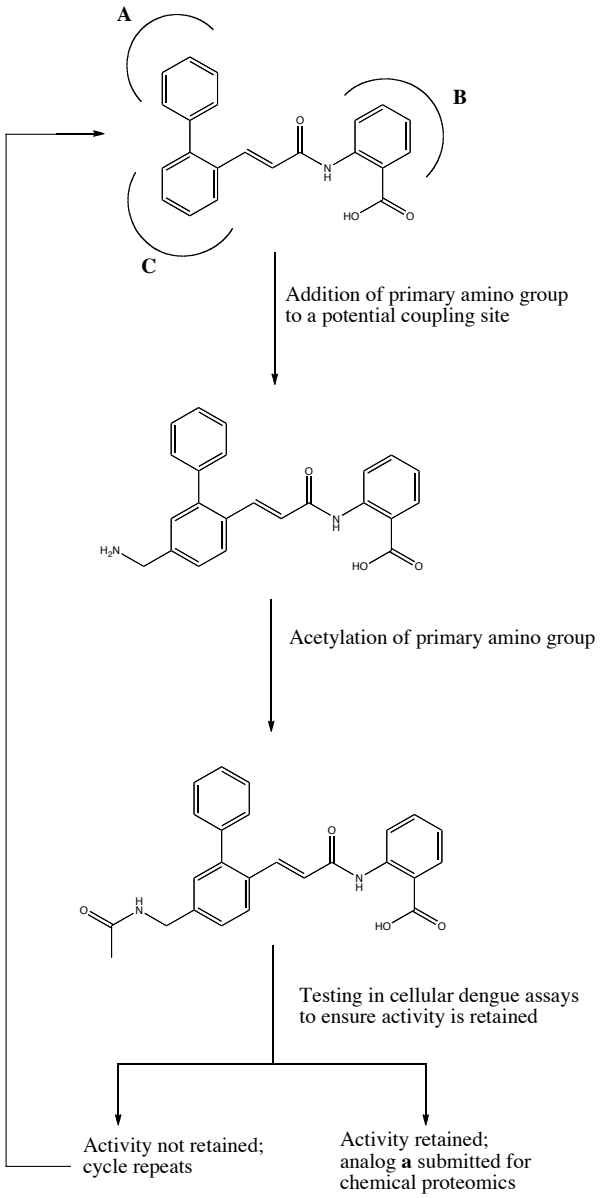


Figure 4 Sequence of events for the design and synthesis of the immobilizable compound

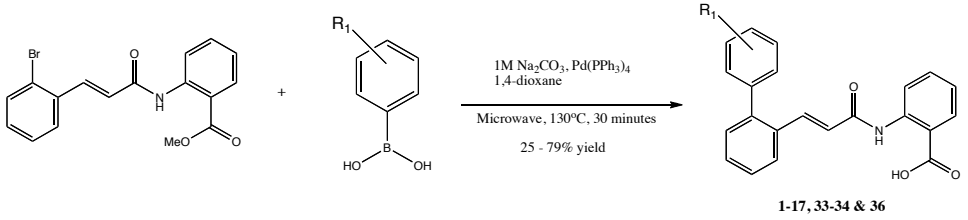
in the cellular dengue assays, analog **a** would be submitted for chemical proteomics analysis.

2.1. Synthesis of analogs of NITD10

Thirty-seven analogs were synthesized and tested in the cellular dengue assays. The design and biological activities of these analogs were the main focus of this study. Therefore, as long as the reactions yielded the desirable compounds, they were not further optimized to give better yields.

2.1.1. Structural modifications at site A

Synthesis of compounds **1-17**, **33-34** & **36** were achieved by coupling commercially available substituted arylboronic acids with a common intermediate, 2-[(E)-3-(2-bomo-phenyl)-acryloylamino]-benzoic acid methyl ester, using a palladium-catalyzed and microwave-assisted Suzuki reaction (Figure 5). The reaction was carried out under basic conditions and 1,4-dioxane was used as the solvent. These reactions proceeded with moderate to good yields (refer to experimental sections).



1: R_1 = 4-CH_3
2: R_1 = 4-$C(CH_3)_3$
3: R_1 = 5-CF_3
4: R_1 = 4-OCH_3
5: R_1 = 4-$N(CH_3)_2$
6: R_1 = 4,5-OCH_3
7: R_1 = 4-CF_3
8: R_1 = 4-F
9: R_1 = 4-CH_2OH
10: R_1 = 2-CH_3
11: R_1 = 3-CH_3
12: R_1 = 3- NH_2, 4-CH_3
13: R_1 = 3-$NHCOCH_3$, 4-CH_3
14: R_1 = 4-$(CH_2)_3CH_3$
15: R_1 = 2-CH_3, 6-CH_3
16: R_1 = 4-NO_2
17: R_1 = 3-$NHCOOC(CH_3)_3$
33: R_1 = 4-$C(CH_3)_2CH_2NH_2$
34: R_1 = 4-$(CH_2)_2NO_2$
36: R_1 = 4-$(CH_2)_2NH_2$

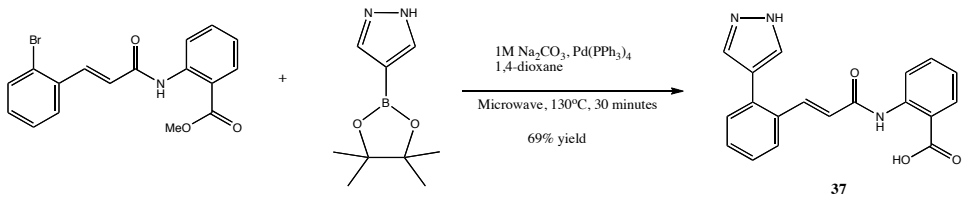
Figure 5 Synthetic scheme for compounds 1-17, 33-34 & 36

Previous reports indicated that even though steric hindrance of aryl halides is not a major factor for the generation of substituted biaryls, low yields are obtained when *ortho*-di-substituted arylboronic acids are used for the coupling (13). When compared to compound **11**,

which was coupled from an arylboronic acid that has a methyl group substituted at the *meta* position and has a yield of 70%, a lower yield of 48% was indeed observed with the synthesis of compound **15** as the arylboronic acid it was coupled from has bulky groups at both *ortho* positions that might hinder the transmetalation step of the coupling cycle.

Electron-withdrawing or electron-donating substituents on the arylboronic acids used in this study did not exert a significant effect on the coupling reaction. No association of the yields with electronic effects was observed. For example, compound **16**, with a nitro substituent, had a yield of 46% and compound **4**, with a methoxy substituent, had a similar yield of 47%. Huang et al (14) reported similar findings indicating that electronic factors of the aromatic halides were significant while electronic effects of the arylboronic counterparts were negligible.

Coupling of 2-[(E)-3-(2-bomo-phenyl)-acryloylamino]-benzoic acid methyl ester with 4-pyrazoleboronic acid pinacol ester (Figure 6) using the same Suzuki conditions mentioned above also gave compound **37** in good yield (69%). This could indicate that various functional groups could be tolerated on the arylboronic acids used for coupling under these conditions.



37

Figure 6 Synthetic scheme for compound 37

Compounds **29** and **30** (Figure 7) were originally Boc-protected when retrieved from the Novartis compound archive. Boc-deprotection with trifluoroacetic acid (TFA) yielded both compounds, as TFA salts, in excellent yields. Subsequent acetylation of both compounds with acetic anhydride and catalytic amounts of triethylamine provided compounds **31** and **32** (Figure 7). These acetylation conditions were also employed for the synthesis of compound

35 from compound **33** (Figure 8). The use of catalytic amounts of base was not ideal and probably explains the poor yields of the acetylation reactions.

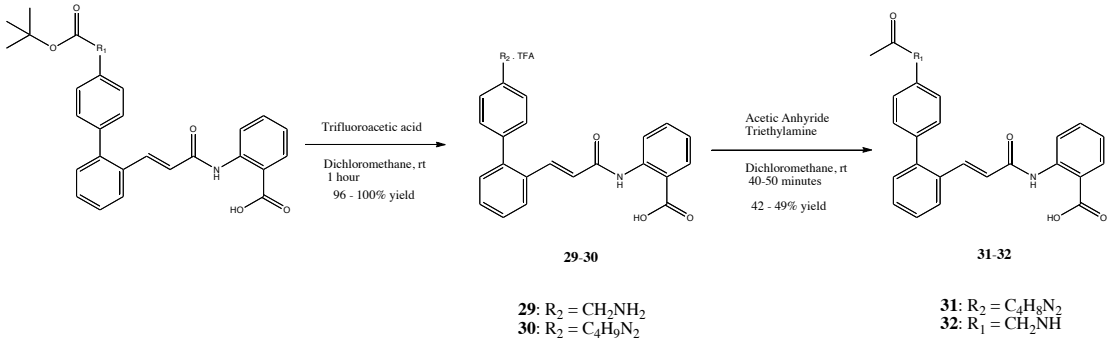


Figure 7 Synthetic scheme for compounds 29-32

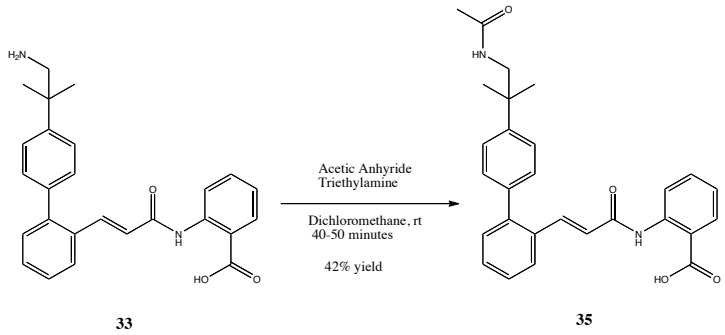


Figure 8 Synthetic scheme for compound 35

2.1.2.Structural modifications at site B

Previous work in our laboratory has shown that the carboxylic acid at site B is essential for activity and could not be a coupling site. Thus, it was left intact in the analogs synthesized.

Intermediates **18-21a** were obtained by esterification of the corresponding benzoic acids with trimethylsilyl diazomethane (Figure 9). These intermediates were subsequently coupled with (E)-3-biphenyl-2-yl-acrylic acid chloride to give compounds **18**, **20**, **21** and intermediate **19b** (Figure 10). Pyridine and dimethylaminopyridine (DMAP) were utilized as catalysts and dimethylformamide (DMF) was used as the solvent. As the methyl ester group of **19b** did not hydrolyze during the microwave process, it was subsequently hydrolyzed with 4M lithium hydroxide (LiOH) in a mixture of tetrahydrofuran and water. The yields of the

amide coupling reactions were generally low, and were due to decomposition of the reaction mixtures under the microwave conditions of 130ºC for about 5-6 hours. Such conditions were necessary for the reactions to proceed.

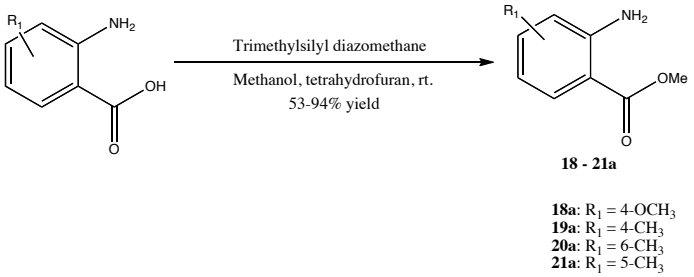


Figure 9 Synthetic scheme for intermediates 18-21a

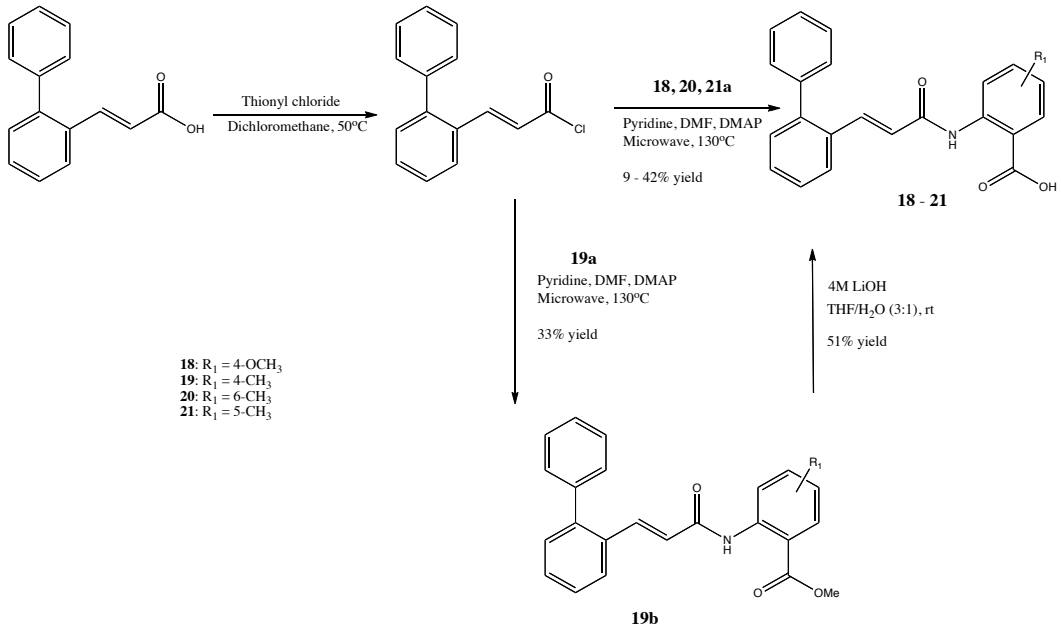


Figure 10 Synthetic scheme for compounds 18-21

Compound **26** was synthesized under the same conditions as compound **19** (Figure 11) and a low yield was also observed in the amide coupling step due to decomposition of reaction mixtures under the harsh microwave conditions.

As shown in Figure 12, amide coupling of (E)-3-biphenyl-2-yl-acrylic acid chloride with commercially available methyl-3-amino-4-nitrobenzoate gave compound **27** in moderate yield (50%). Subsequent reduction of the nitro group of compound **27** with tin (II) chloride dihydrate yielded compound **28**.

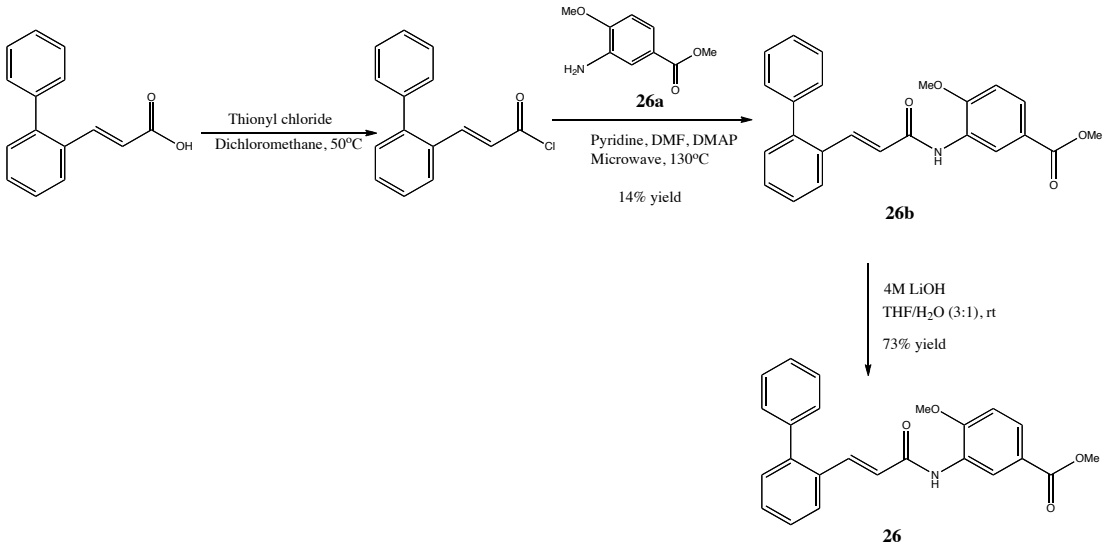


Figure 11 Synthetic scheme for compound 26

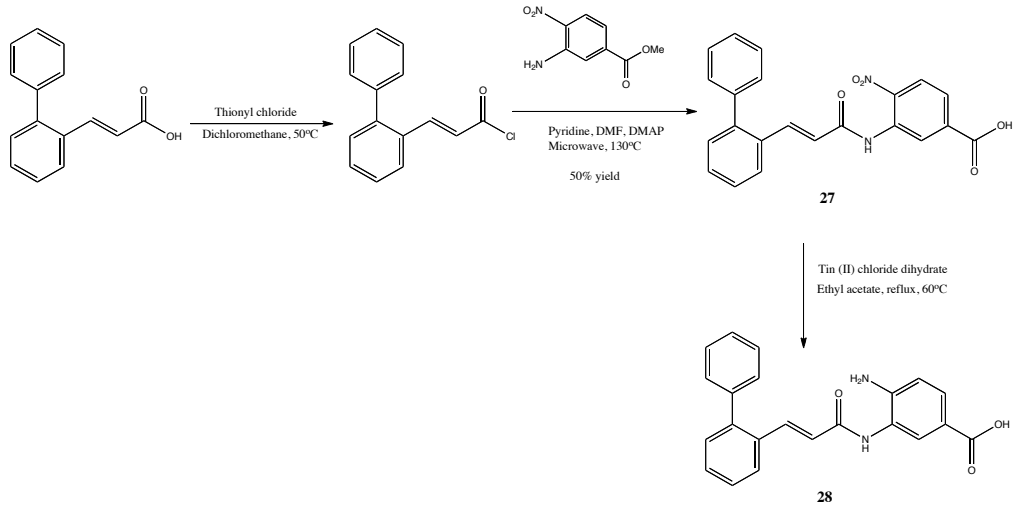


Figure 12 Synthetic scheme for compounds 27-28

2.1.3.Structural modifications at site C

The synthetic scheme for compounds **22-25** is illustrated in Figure 13. Intermediate **22a** was prepared from commercially available 2-bromo-4-nitrobenzaldehyde and methyl diethyl phosphonoacetate by a Horner-Wadsworth Emmons reaction. Butyllithium (BuLi) was used as a base and tetrahydrofuran (THF) was used as the solvent. Hydrolysis of the methyl ester in intermediate **22a**, using 4M LiOH, provided intermediate **22b**. The acid chloride of intermediate **22b** was prepared using thionyl chloride. The amide coupling reaction between the acid chloride and methyl anthranilate used pyridine and DMAP as

catalysts and DMF as solvent and was stirred at room temperature for 20 hours. This milder condition, as compared to the microwave conditions, provided intermediate **d** with an improved yield of 60%. Suzuki coupling of intermediate **d** with phenylboronic acid or 4-tert-butylphenylboronic acid yielded compounds **22** and **23** respectively. Subsequent reduction of the nitro groups in compounds **22** and **23** provided compounds **24** and **25** respectively.

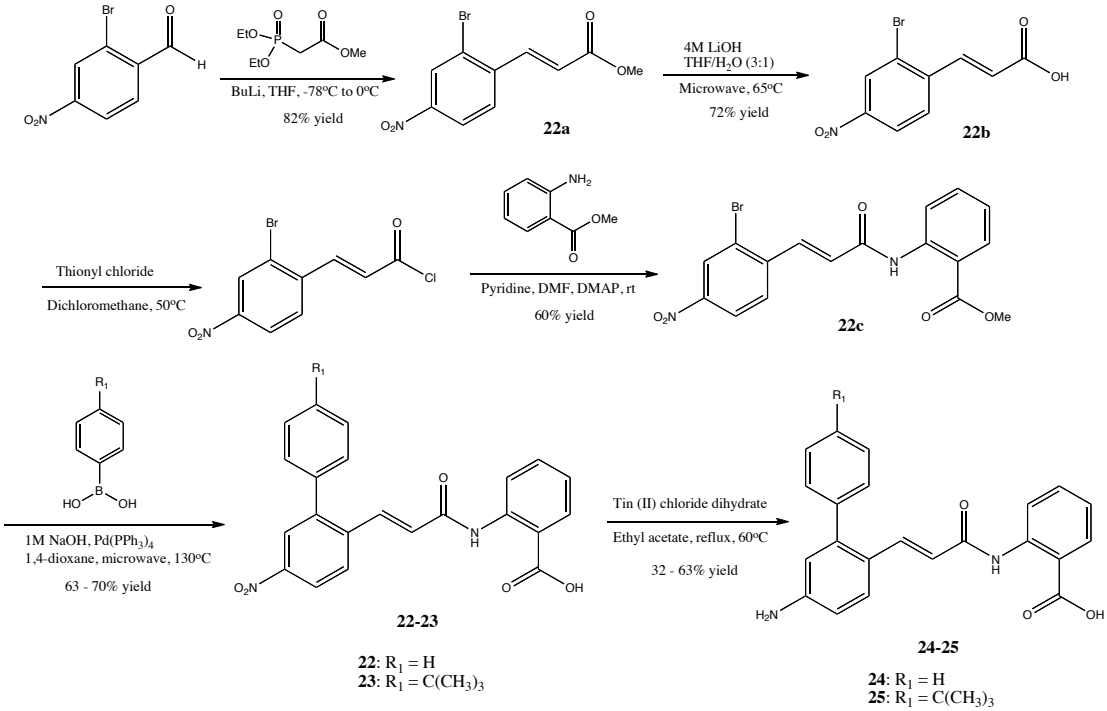


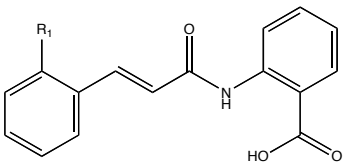
Figure 13 Synthetic scheme for compounds 22-25

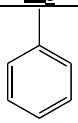
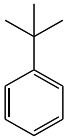
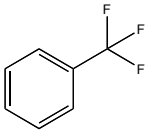
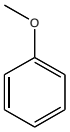
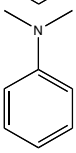
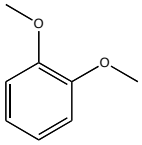
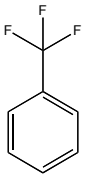
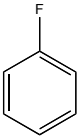
2.2. Biological testing of analogs

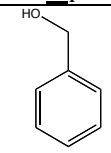
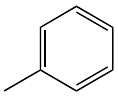
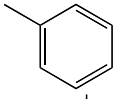
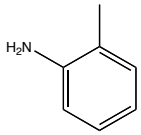
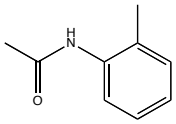
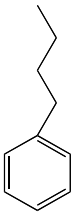
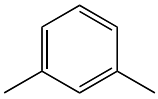
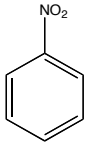
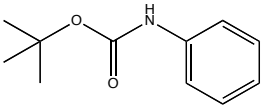
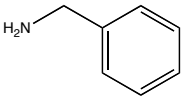
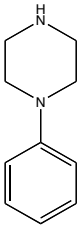
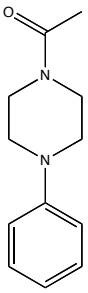
Compounds **1-58** (synthesis of compounds **38-56** was performed by a contract research organization, GVK Biosciences and compounds **57-58** were synthesized in-house previously) were evaluated for their *in-vitro* anti-viral acitivity in the in-house cell-based flavivirus immunodetection (CFI) assay. This assay is based on quantitative immunodetection of viral envelope protein, E, as a readout for viral load in target cells. Both the IC_{50} and CC_{50} (half maximal cytotoxic concentration) values were determined to distinguish anti-viral activity from cytotoxcitiy.

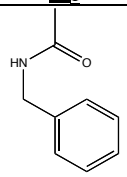
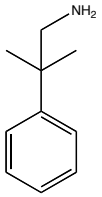
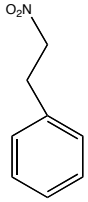
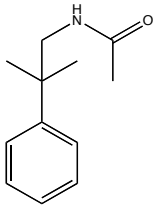
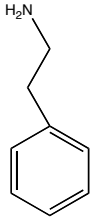
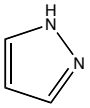
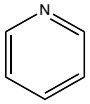
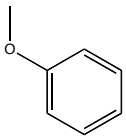
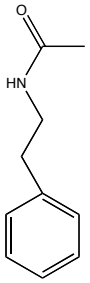
2.2.1. Evaluation of analogs with modifications at site A

Site A is amendable to modifications and several analogs have desirable activities. However, these analogs did not have a primary amino group in their scaffold for immobilization onto the solid matrix. On the other hand, potential immobilizable compounds, with their primary amino group acetylated, did not retain their anti-viral activity (Table 1).



Compound	R_1	IC_{50} (µM)	CC_{50} (µM)
1		0.4	47.5
2		0.4	31.6
3		2.9	34.3
4		1.5	38.0
5		1.4	50.6
6		12.9	34.5
7		0.7	18.3
8		1.3	26.6

Compound	R_1	IC_{50} (µM)	CC_{50} (µM)
9		15.5	33.8
10		0.4	13.5
11		0.4	18.3
12		6.8	27.2
13		**45.6**	**68.1**
14		2.89	31.1
15		1.01	42.3
16		1.4	17.1
17		5.4	19.6
29		10.8	>100
30		55.5	>100
31		**31.3**	**30.0**

Compound	R_1	IC_{50} (μM)	CC_{50} (μM)
32		**43.4**	**70.4**
33		19.9	>100
34		4.7	14.2
35		**11.4**	**22.1**
36		1.2	56.1
37		1.3	65.1
47		52.5	60.2
48		24.4	72.3
49		3.2	67.6
51		**35.9**	**70.8**

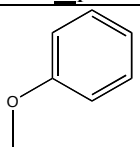
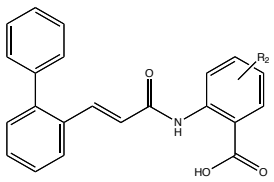
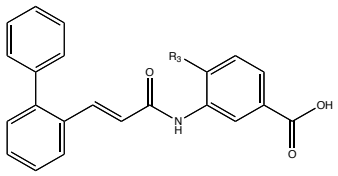
Compound	R_1	IC_{50} (µM)	CC_{50} (µM)
55		2.0	74.5

Table 1 Biological activities of compounds with modifications at site A (activities of potential immobilized compounds are highlighted in bold)

2.2.2. Evaluation of analogs with modifications at site B

Site B does not tolerate changes and most of the analogs synthesized do not have the desired anti-viral activity in the CFI assay. Compound **57** is an exception and showed moderate *in-vitro* anti-viral activity (Table 2).

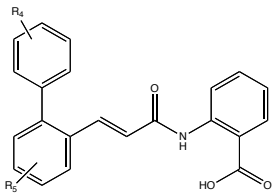




Compound	R_2	R_3	IC_{50} (µM)	CC_{50} (µM)
18	4-OCH_3	--	13.6	42.0
19	4-CH_3	--	50.5	78.6
20	6-CH_3	--	36.2	68.6
21	5-CH_3	--	10.0	48.0
26	--	OCH_3	27.6	>100
27	--	NO_2	24.3	51.4
28	--	NH_2	>100	>100
42	**5-$NHCOCH_3$**	**--**	**46.3**	**>100**
43	5-NH_2	--	74.8	74.2
44	4-NH_2	--	33.5	72.1
45	5-CH_2NH_2	--	51.7	>100
46	**5-$CH_2NHCOCH_3$**	**--**	**>100**	**>100**
56	**4-$NHCOCH_3$**	**--**	**>100**	**>100**
57	4-Cl	--	2.5	32.3
58	5-Cl	--	12.0	34.8

Table 2 Biological activities of compounds with modifications at site B (activities of potential immobilized compounds are highlighted in bold)

2.2.3. Evaluation of analogs with modifications at site C



Compound	R_4	R_5	IC_{50} (μM)	CC_{50} (μM)
22	H	5-NO_2	0.7	>100
23	4-$C(CH_3)_3$	5-NO_2	1.5	19.6
24	H	5-NH_2	10.8	>100
25	4-$C(CH_3)_3$	5-NH_2	0.8	57.6
38	H	6-NH_2	13.9	54.9
39	H	4-NH_2	22.1	60.8
40	**H**	**4-$NHCOCH_3$**	**>100**	**>100**
41	**H**	**6-$NHCOCH_3$**	**93.6**	**72.0**
50	H	4-CH_2NH_2	>20	>20
52	**H**	**4-$CH_2NHCOCH_3$**	**>100**	**>100**
53	**H**	**5-$CH_2NHCOCH_3$**	**>100**	**>100**
54	H	5-CH_2NH_2	>100	>100

Table 3 Biological activities of compounds with modifications at site C (activities of potential immobilized compounds are highlighted in bold)

As shown in Table 3, site C is also not as amendable to changes as site A. When primary amino groups were introduced, only compound **25** had an IC_{50} of less than 1.0μM. However, once the primary amino groups were acetylated, all inhibitory activity in the CFI assay was lost.

3. Discussion

3.1. Search for the immobilizable compound

The design and synthetic efforts carried out in this study were unable to yield an immobilizable compound for the target pull down of NITD10 using the chemical proteomics technology. Several reasons could be attributed to it.

Being a small molecular weight compound, NITD10 (molecular weight of 343) could possibly be embedded in its target and majority of the scaffold has key interactions with the target. Thus, locating a site, which is synthetically accessible, for the immobilization of an analog, would be difficult. Furthermore, this study did not explore the middle region of the scaffold where the double bond and amide bond reside. This region could be a potential site and more research has to be performed to explore such a possibility.

NITD10 could also be a non-specific cytotoxic agent and have interactions with multiple targets. A site that does not have major interactions with one target might form important bonds with another target. In this scenario, the chance of finding a site that does not have any key interactions with any of its targets would be low.

3.2. Structure activity relationships of NITD10 with its target

This study explored some of the interactions of NITD10 with its target. The SAR will be presented in the subsequent paragraphs.

3.2.1. Interactions at site A

Compounds that contain alkyl groups, in the form of a single methyl group (compound **1**, **10** and **11**) or a *t*-butyl group (compound **2**), have improved activities in the CFI assay as compared to NITD10. This suggests that there are important hydrophobic interactions between these alkyl groups and a non-polar surface area of the target. The interaction of these two non-polar surfaces reduces the amount of structured water at the interface and thus, provides a favorable entropy of association. The overall strength of the hydrophobic interaction is very dependent on the quality of the steric match between the two

surfaces (15) and this was demonstrated in compounds **2** and **14**. Both compounds contain the same number of carbons in their alkyl side chains, but compound **2**, being a compact molecule, is more active than compound **14**, which has an extended *n*-butyl chain substituted.

The presence of phenyl rings could lead to possible π-π interactions between the phenyl ring and an electron-rich area on the target. It was postulated that by rendering the phenyl ring more electron-deficient with the presence of electron-withdrawing groups, such as CF_3, NO_2 and F, it could achieve a stronger π-π interaction with the electron dense region (Figure 14). On the other hand, when electron-donating groups, such as OCH_3 and $N(CH_3)_2$, are substituted, the phenyl ring, being more electron rich, would form a weaker interaction with the electron-rich region and the activity of the compound is predicted to decrease. However, experimental results showed that electronic factors do not govern the activity of the compound. Compound **5**, which contains a strong electron-donating N-dimethyl group, exhibited the same activity as compound **16**, which contains a strong electron-withdrawing nitro group. Likewise for compounds **4** and **8**, similar activities were observed for both even though compound **4** has an electron-donating methoxy group substituted and compound **8** contains an electron-withdrawing fluoride.

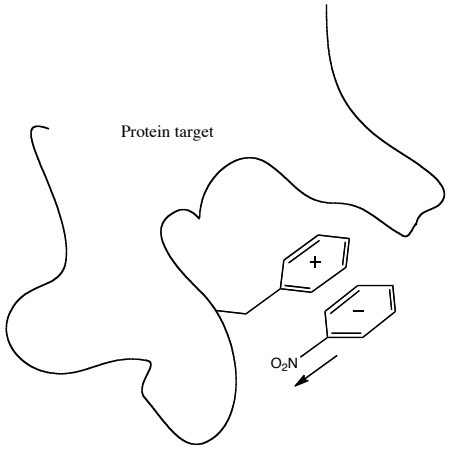


Figure 14 A simple illustration of a possible π-π interaction between the electron-deficient phenyl ring and an electron-rich area on the target

The introduction of a primary amino group at site A did not favor the interaction of the compound with its target. As seen from compounds **12**, **29** and **3**, there were only

moderate activities observed in the CFI assays. Acetylation of these primary amines caused a further decrease in the *in-vitro* anti-viral activities. This was reflected in compounds **13**, **32** and **51** respectively.

In summary, the optimal substituent at site A could simply be a single methyl group, which could introduce favorable hydrophobic interactions between the compound and its target.

3.2.2. Interactions at site B

As seen from the biological activities of analogs with modifications at site B, this part of the scaffold does not tolerate changes and activity was lost when substitutions were made. A possible explanation could be that site B has very specific interactions with the target and any modification that could alter the steric match between site B and the target would reduce the strength of the interaction. However, anti-viral activity was observed with compound **57**, which has a chloro group in the 4-position of the phenyl ring at site B and an IC_{50} of 2.5 μM. When the chloro group was substituted with electron-donating groups, such as methoxy (compound **18**) and methyl (compound **19**), a decrease in activity was observed. This could suggest the presence of electronic effects.

3.2.3. Interactions at site C

The modifications at site C did not explore much of the SAR between NITD 10 and its target and were focused on identifying a linking site. With compound **25**, it was designed to include a non-polar moiety, namely a *t*-butyl group, at site A in an attempt to utilize the favorable hydrophobic interactions to improve the overall binding affinity for the target. It has a desirable activity of an IC_{50} of 0.8 μM but once the primary amino group was acetylated, the activity was lost. Substitution of a primary amino group on the phenyl ring at site C is most optimal at the 5-position of the ring. However, when the primary amino group at 5-position was extended with a methylene group (compound **54**), activity was lost. Similar to site B, this could indicate specific interactions that do not accommodate steric bulk.

4. Conclusion

In this study, an attempt was made to synthesize an immobilizable compound for the target identification of NITD10 using chemical proteomics. However, all analogs with the acetylated primary amino group incorporated did not have the desired activity in the CFI assay and thus, the chemical proteomics analysis was not carried out. Possible interactions for NITD10 with its target(s) were highlighted and the suitability of NITD10 as a tool compound for the target pull down experiment was questioned.

Thirty-seven analogs were synthesized and even though an immobilizable compound was not found, a preliminary SAR was established. Site A proved to be most amendable to changes and analogs of NITD10 with improved activity have been found. On the other hand, sites B and C were less accommodating to modifications. These findings could provide useful information for the future optimization of NITD10 once its target has been identified.

5. Experimental Sections

5.1. General Methods

Materials and reagents used were of the highest commercially available grade and without further purification. 2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester and (E)-3-biphenyl-2-yl-acrylic acid were obtained from the chemical archive of NITD.

Reactions requiring microwave irradiation were performed on a Biotage Initiator™ microwave system with an operating frequency of 2.4 GHz.

Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} (2.5 × 7.5 cm) using UV light (λ = 254nm) for visualization. TLC data are given as the R_f value with the corresponding eluent system specified in brackets.

^{1}H NMR and ^{13}C NMR spectra were determined on a Varian 300 Mercury spectrometer. Chemical shifts (δ) are expressed in ppm. Splitting patterns are described as singlet (s), broad singlet (br.s.), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), doublet of triplet (dt), triplet (t) or multiplet (m).

LC-MS analyses were performed with an Agilent LC1100 coupled with Applied Biosystems API2000, using the following conditions: monolithic-C18, 50 × 4.6 mm column; mobile system of acetonitrile/water with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 4 min; UV 254 and 214 nm; and flow rate of 3 mL/min.

HPLC purity determinations were made on an Agilent LC1100 HPLC, using the following conditions: SymmetryShield RP-18 3.5µm, 150 × 4.6 mm column; mobile system of acetonitrile/water with 0.1% of formic acid with a gradient of 5-95% acetonitrile; run time of 20 min; UV 254 and 214 nm; and flow rate of 0.8 mL/min.

Flash chromatography was performed using a Teledyne ISCO CombiFlash® system using RediSep® Rf disposable Flash columns. HPLC purification was carried out on a Waters Prep LC with an Atlantis C18, 10µm, 19 × 250 mm column; mobile system of acetonitrile/water with 0.1% of formic acid; run time of 40 min; UV 254 and 214 nm; and flow rate of 20 mL/min.

5.2. General Procedures

5.2.1. General procedure for the Suzuki coupling of 2-[(E)-3-(2-bromo-phenyl)-acryloylamino]-benzoic acid methyl ester with a boronic acid

2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (1.0 eq), boronic acid (1.0 eq) and tetrakis(triphenylphosphine) palladium (0) (0.05 eq) were dissolved in dry 1,4-dioxane (1 mL) to form a clear yellow solution. 1M Na_2CO_3 (3.0 eq) was subsequently added and the resulting mixture, with white precipitate, was subjected to microwave irradiation at 130ºC for 30 minutes. Purification of the crude reaction mixture by HPLC yielded the benzoic acid.

5.2.2. General procedure for esterification of benzoic acid

Benzoic acid (1.0 eq) was dissolved in dry tetrahydrofuran and trimethylsilyl diazomethane (1.6 eq) and dry methanol were added subsequently. The reaction mixture was stirred at room temperature, under argon, to completion. A few drops of acetic acid were added to quench the reaction, which was further evaporated to give a crude mixture. Purification of the crude mixture by flash chromatography yielded the benzoic acid methyl ester.

5.2.3. General procedure for reduction of a nitro group

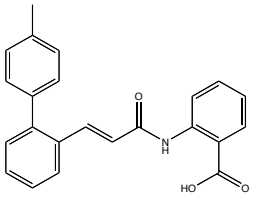
The nitro compound (1.0 eq) was dissolved in ethyl acetate (2.5 mL) and tin (II) chloride dihydrate (3.0-4.0 eq) was added subsequently. The resulting mixture was refluxed at 60^0C for 23 hours. Upon cooling, the reaction mixture was quenched with cold saturated sodium bicarbonate solution (30 mL) and extracted with ethyl acetate (3 x 20 mL) with solid sodium chloride added. The organic layers were combined, dried over sodium sulfate and concentrated. Purification of the crude mixture by HPLC yielded the reduced amino compound.

5.2.4. General procedure for Boc-deprotection

The Boc-protected amine was added to a mixture of trifluoroacetic acid (TFA) (0.6 mL), dichloromethane (1.4 mL) and a few drops of water. The reaction mixture was stirred at room temperature for 1 hour. After the reaction mixture was concentrated, the residue was triturated with ether to obtain the TFA-salt.

5.3. Synthesis of analogs of NITD10

2-[(E)-3-(4'-Methyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (1)



2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (86.5 mg, 0.24 mmol) was coupled with 4-tolylboronic acid (32.6 mg, 0.24 mmol) following the general procedure. The obtained bright yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (30.8 mg, 0.09 mmol, 36% yield).

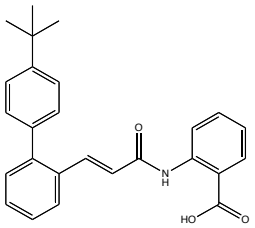
LC-MS: R_t 2.90 mins; m/z (ESI): [M+H]+ 358; [M-H]- 356.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 2.38 (s, 3H, CH_3), 6.55 (d, *J* = 15.0 Hz, 1H, CH=CH), 7.13 (dt, *J* = 6.3, 2.7 Hz, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 7.38-7.47 (m, 6H, Ar-H), 7.61 (dt, *J* = 6.0, 2.4 Hz, 1H, Ar-H), 7.74-7.79 (m, 1H, Ar-H), 7.84 (d, *J* = 15.3, 1H, CH=CH), 8.13 (dd, *J* = 7.2, 2.7 Hz, 1H, Ar-H), 8.86 (dd, *J* = 9.0, 2.4 Hz, 1H, Ar-H), 11.12 (br.s., 1H, NH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.31, 115.40, 121.04, 122.60, 123.06, 127.04, 127.61, 129.29 (2C), 129.90 (2C), 129.99, 130.81, 132.08, 132.93, 135.82, 137.17, 137.56, 142.46, 142.53, 143.36, 165.15, 172.36.

Purity: >95% by HPLC.

2-[(E)-3-(4'-tert-Butyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (2)



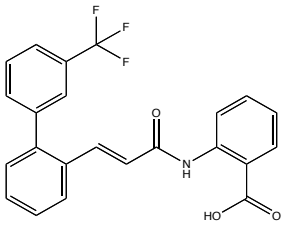
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (80.0 mg, 0.22 mmol) was coupled with 4-tert-butylphenylboronic acid (39.5 mg, 0.22 mmol) following the general procedure. The obtained yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white crystalline solid (49.3 mg, 0.12 mmol, 56% yield).

LC-MS: R_t 3.26 mins; m/z (ESI): [M+H]+ 400; [M-H]- 398.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 1.36 (s, 9H, $(CH_3)_3$), 6.56 (d, *J* = 15.6 Hz, 1H, CH=CH), 7.11 (dt, *J* = 7.2, 3.0 Hz, 1H, Ar-H), 7.25-7.30 (m, 2H, Ar-H), 7.35-7.47 (m, 5H, Ar-H), 7.58 (dt, *J* = 6.0, 3.2 Hz, 1H, Ar-H), 7.74-7.78 (m, 1H, Ar-H), 7.87 (d, *J* = 15.3, 1H, CH=CH), 8.12 (dd, *J* = 6.2, 3.0 Hz, 1H, Ar-H), 8.84 (dd, *J* = 9.0, 2.4 Hz, 1H, Ar-H), 11.10 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(3'-Trifluoromethyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (3)



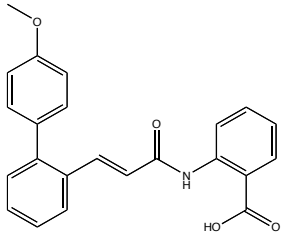
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 3-trifluoromethylphenylboronic acid (31.6 mg, 0.17 mmol) following the general procedure. The obtained pale yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (32.8 mg, 0.08 mmol, 48% yield).

LC-MS: R_t 2.93 mins; m/z (ESI): [M+H]+ 412; [M-H]- 410.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 6.73 (d, J = 15.0Hz, 1H, CH=CH), 7.15 (dt, J = 6.0, 3.2 Hz, 1H, Ar-H), 7.39-7.45 (m, 1H, Ar-H), 7.50-7.77 (m, 8H, Ar-H & CH=CH), 7.88-7.92 (m, 1H, Ar-H), 8.11 (dd, J = 9.0, 3.0 Hz, 1H, Ar-H), 8.62 (dd, J = 9.0, 2.4 Hz, 1H, Ar-H).

Purity: >99% by HPLC.

2-[(E)-3-(4'-Methoxy-biphenyl-2-yl)-acryloylamino]-benzoic acid (4)



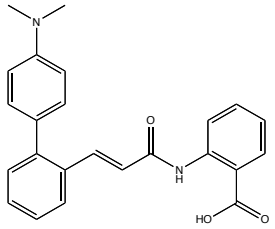
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 4-methoxybenzeneboronic acid (25.3 mg, 0.17 mmol) following the general procedure. The obtained pale yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (29.1 mg, 0.08 mmol, 47% yield).

LC-MS: R_t 2.71 mins; m/z (ESI): [M+H]+ 374; [M-H]- 372.

^{1}H NMR (300 MHz, $CDCl_3$-d): δ = 3.91 (s, 3H, O-CH_3), 6.53 (d, J = 15.0Hz, 1H, CH=CH), 6.93-6.97 (m, 2H, Ar-H), 7.11 (dt, J = 8.0, 1.2 Hz, 1H, Ar-H), 7.26-7.28 (m, 2H, Ar-H), 7.32-7.42 (m, 3H, Ar-H), 7.58 (dt, J = 7.0, 2.0 Hz, 1H, Ar-H), 7.71-7.75 (m, 1H, Ar-H), 7.81 (d, J = 15.0Hz, 1H, CH=CH), 8.11 (dd, J = 9.0, 3.0 Hz, 1H, Ar-H), 8.84 (dd, J = 9.0, 2.4 Hz, 1H, Ar-H), 11.10 (br.s., 1H, NH).

Purity: >95% by HPLC.

2-[(E)-3-(4'-Dimethylamino-biphenyl-2-yl)-acryloylamino]-benzoic acid (5)



2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (50.0 mg, 0.14 mmol) was coupled with 4-dimethylaminophenylboronic acid (22.9 mg, 0.14 mmol) following the

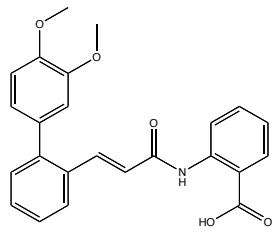
general procedure. The obtained bright orange yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (20-95%) in water. The title compound was isolated as a yellow solid (24.1 mg, 0.06 mmol, 45% yield).

LC-MS: R_t 2.56 mins; m/z (ESI): [M+H]+ 387; [M-H]- 385.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.98 (s, 6H, N-$(CH_3)_2$), 6.77-6.85 (m, 3H, CH=CH & Ar-H), 7.12-7.19 (m, 3H, Ar-H), 7.33-7.49 (m, 3H, Ar-H), 7.58 (dt, *J* = 7.5, 2.7 Hz, 1H, Ar-H), 7.67 (d, *J* = 14.4, 1H, CH=CH), 7.90 (dd, *J* = 8.1, 2.7 Hz, 1H, Ar-H), 8.00 (dd, *J* = 8.4, 3.0 Hz, 1H, Ar-H), 8.55 (dd, *J* = 8.2, 2.7 Hz, 1H, Ar-H), 11.48 (br.s., 1H, NH).

Purity: >95% by HPLC.

2-[(E)-3-(3', 4'-Dimethoxy-biphenyl-2-yl)-acryloylamino]-benzoic acid (6)



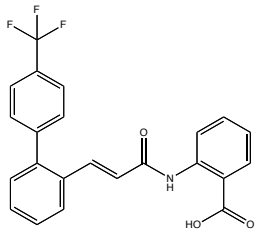
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (50.0 mg, 0.14 mmol) was coupled with 3,4-dimethoxyphenylboronic acid (25.3 mg, 0.14 mmol) following the general procedure. The obtained bright yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as an off-white solid (29.6 mg, 0.07 mmol, 53% yield).

LC-MS: R_t 2.55 mins; m/z (ESI): [M+H]+ 404; [M-H]- 402.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 3.86 (s, 3H, O-CH_3), 3.91 (s, 3H, O-CH_3), 6.54 (d, *J* = 15.6 Hz, 1H, CH=CH), 6.85-6.95 (m, 3H, Ar-H), 7.10 (dt, *J* = 6.6, 2.7 Hz, 1H, Ar-H), 7.33-7.44 (m, 3H, Ar-H), 7.58 (dt, *J* = 8.7, 3.0 Hz, 1H, Ar-H), 7.71-7.76 (m, 1H, Ar-H), 7.80 (d, *J* = 15.3 Hz, 1H, CH=CH), 8.09 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar-H), 8.82 (dd, *J* = 9.0, 1.2 Hz, 1H, Ar-H), 11.19 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(4'-Trifluoromethyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (7)



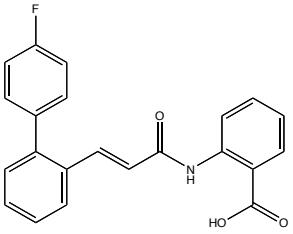
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (50.0 mg, 0.14 mmol) was coupled with 4-trifluoromethylphenylboronic acid (26.4 mg, 0.14 mmol) following the general procedure. The obtained bright yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (45.0 mg, 0.11 mmol, 79% yield).

LC-MS: R_t 2.98 mins; m/z (ESI): [M+H]+ 412; [M-H]- 410.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 6.75 (d, *J* = 14.4, 1H, CH=CH), 7.15 (dt, *J* = 6.9, 2.7 Hz, 1H, Ar-H), 7.39-7.45 (m, 1H, Ar-H), 7.49-7.58 (m, 5H, Ar-H), 7.66 (d, *J* = 15.0 Hz, 1H, CH=CH), 7.77-7.82 (m, 2H, Ar-H), 7.89-7.93 (m, 1H, Ar-H), 8.11 (dd, *J* = 8.7, 1.5 Hz, 1H, Ar-H), 8.61 (dd, *J* = 7.8, 2.7 Hz, 1H, Ar-H).

Purity: >99% by HPLC.

2-[(E)-3-(4'-Fluoro-biphenyl-2-yl)-acryloylamino]-benzoic acid (8)



2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (50.0 mg, 0.14 mmol) was coupled with 4-fluorophenylboronic acid (19.4 mg, 0.14 mmol) following the general procedure. The obtained yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (28.6 mg, 0.08 mmol, 57% yield).

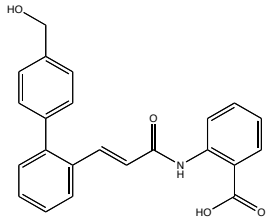
LC-MS: R_t 2.79 mins; m/z (ESI): [M+H]+ 362; [M-H]- 360.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 6.51 (d, *J* = 14.7 Hz, 1H, CH=CH), 7.01-7.12 (m, 3H, Ar-H), 7.25-7.32 (m, 3H, Ar-H), 7.34-7.41 (m, 2H, Ar-H), 7.46-7.52 (m, 1H, Ar-H), 7.64-7.71

(m, 2H, CH=CH & Ar-H), 8.04 (dd, *J* = 9.0, 3.0 Hz, 1H, Ar-H), 8.71 (dd, *J* = 9.0, 2.4 Hz, 1H, Ar-H), 11.53 (br.s., 1H, NH).

Purity: >98% by HPLC.

2-[(E)-3-(4'-Hydroxymethyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (9)



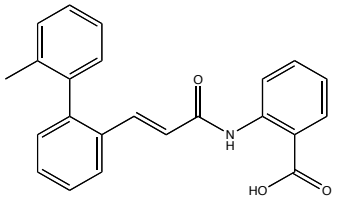
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (50.0 mg, 0.14 mmol) was coupled with 4-hydroxymethylphenylboronic acid (21.1 mg, 0.14 mmol) using the general procedure. The obtained yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (19.9 mg, 0.05 mmol, 38% yield).

LC-MS: R_t 2.32 mins; m/z (ESI): [M-H]- 372.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 4.69 (s, 2H, CH_2), 6.67 (d, *J* = 15.0 Hz, 1H, CH=CH), 7.15 (dt, *J* = 9.0, 3.0 Hz, 1H, Ar-H), 7.31-7.58 (m, 8H, Ar-H), 7.73 (d, *J* = 15.3 Hz, 1H, CH=CH), 7.84-7.87 (m, 1H, Ar-H), 8.10 (dd, *J* = 9.0, 3.0 Hz, 1H, Ar-H), 8.62 (dd, *J* = 9.0, 2.7 Hz, 1H, Ar-H).

Purity: >98% by HPLC.

2-[(E)-3-(2'-Methyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (10)



2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 2-tolylboronic acid (22.7 mg, 0.17 mmol) using the general procedure. The obtained bright yellow solution with black precipitate was filtered and purified by HPLC,

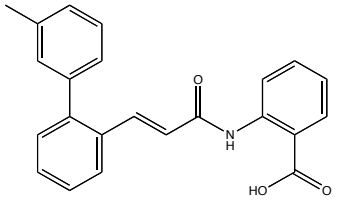
eluting with acetonitrile (30-95%) in water. The title compound was isolated as an off- white solid (22.8 mg, 0.06 mmol, 38% yield).

LC-MS: R_t 2.90 mins; m/z (ESI): [M+H]+ 358; [M-H]- 356.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 2.05(s, 3H, CH_3), 6.62 (d, *J* = 15.0 Hz, 1H, CH=CH), 7.09-7.17 (m, 2H, Ar-H), 7.20-7.33 (m, 4H, Ar-H), 7.40-7.56 (m, 4H, Ar-H & CH=CH), 7.87-7.91 (m, 1H, Ar-H), 8.09 (dd, *J* = 8.1, 3.0 Hz, 1H, Ar-H), 8.57 (dd, *J* = 8.4, 2.4 Hz, 1H, Ar-H).

Purity: >98% by HPLC.

2-[(E)-3-(3'-Methyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (11)



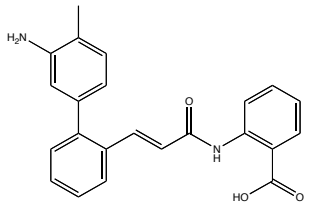
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 3-tolylboronic acid (22.7 mg, 0.17 mmol) using the general procedure. The obtained bright yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (41.7 mg, 0.12 mmol, 70% yield).

LC-MS: R_t 2.94 mins; m/z (ESI): [M+H]+ 358; [M-H]- 356.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 2.41 (s, 3H, CH_3), 6.67 (d, *J* = 15.6 Hz, 1H, CH=CH), 7.09-7.25 (m, 4H, Ar-H), 7.42-7.58 (m, 5H, Ar-H), 7.73 (d, *J* = 15.0 Hz, 1H, CH=CH), 7.83-7.87 (m, 1H, Ar-H), 8.10 (dd, *J* = 8.7, 2.4 Hz, 1H, Ar-H), 8.61 (dd, *J* = 8.7, 2.1 Hz, 1H, Ar-H).

Purity: >99% by HPLC.

2-[(E)-3-(3'Amino-4-methyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (12)



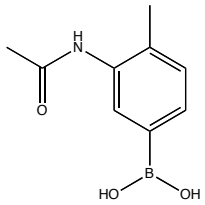
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (80.0 mg, 0.22 mmol) was coupled with 3-amino-4-methylphenylboronic acid (33.5 mg, 0.22 mmol) according to the general procedure. The obtained brown yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (53.2 mg, 0.14 mmol, 72% yield).

LC-MS: R_t 2.34 mins; m/z (ESI): [M+H]+ 373; [M-H]- 371.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.10 (s, 3H, CH_3), 6.38 (dd, *J* = 6.6 Hz, 3.0Hz, 1H, Ar-H), 6.55 (d, *J* = 2.1 Hz, 1H, Ar-H), 6.76 (d, *J* = 15.6 Hz, 1H, CH=CH), 6.99 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.11 (dt, *J* = 7.5, 3.0 Hz, 1H, Ar-H), 7.28 (dd, *J* = 6.3, 2.7 Hz, 1H, Ar-H), 7.36-7.46 (m, 2H, Ar-H), 7.48-7.56 (m, 1H, Ar-H), 7.62 (d, *J* = 15.6 Hz, 1H, CH=CH), 7.89 (dd, *J* = 8.1, 2.1 Hz, 1H, Ar-H), 7.98 (dd, *J* = 7.5, 2.1 Hz, 1H, Ar-H), 8.52 (dd, *J* = 9.0, 1.5 Hz, 1H, Ar-H), 11.85 (br.s., 1H, NH).

Purity: >99% by HPLC.

3-Acetylamino-4-methylphenylboronic acid (13a)

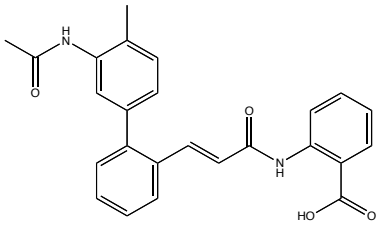


3-Amino-4-methylphenylboronic acid (100.2 mg, 0.66 mmol) was dissolved in pyridine (3 mL) to give a clear brown solution. Acetic anhydride (2 mL) and catalytic amount of 4-dimethylaminopyridine were subsequently added. The reaction mixture was stirred at room temperature for 1 hr. Ethyl acetate (10 mL) was added and the organic layer was washed with 1M hydrochloric acid (10 mL). The organic layer was dried over sodium sulfate and purified with HPLC, eluting with acetonitrile (0-50%) in water. The title compound was isolated as a white solid (42.7 mg, 0.22 mmol, 33% yield).

LC-MS: R_t 0.86 mins; m/z (ESI): [M+H]+ 194; [M-H]- 192.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 2.15 (s, 3H, CH_3), 2.24 (s, 3H, O=C-CH_3), 7.17-7.66 (m, 3H, Ar-H).

2-[(E)-3-(3-Acetyl-amino-4-methyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (13)



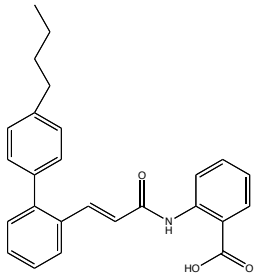
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (69.1 mg, 0.19 mmol) was coupled with 3-acetylamino-4-methylphenylboronic acid **13a** (37.0 mg, 0.19 mmol) according to the general procedure. The obtained dark brown yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (52.1 mg, 0.13 mmol, 66% yield).

LC-MS: R_t 2.32 mins; m/z (ESI): [M+2H]+ 416; [M-H]- 413.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.08 (s, 3H, CH_3), 2.28 (s, 3H, O=C-CH_3), 6.82 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.01 (dd, *J* = 7.77, 1.61 Hz, 1H, Ar-H), 7.14 (ddd, *J* = 8.06, 7.18, 1.17 Hz, 1H, Ar-H), 7.29-7.36 (m, 2H, Ar-H), 7.41-7.61 (m, 5H, Ar-H & CH=CH), 7.95 (dd, *J* = 7.62, 1.76 Hz, 1H, Ar-H), 7.99 (dd, *J* = 7.91, 1.47 Hz, 1H, Ar-H), 8.52 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 9.50 (br.s., 1H, NH), 11.65 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(4'-Butyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (14)



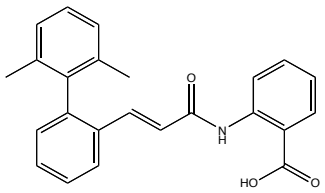
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 4-butylphenyl boronic acid (29.7 mg, 0.17 mmol) according to the general procedure. The obtained pale green yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (50-95%) in water. The title compound was isolated as a white solid (44.5 mg, 0.11 mmol, 67% yield).

LC-MS: R_t 3.40 mins; m/z (ESI): [M-H]- 398.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 0.97 (t, *J* = 7.33 Hz, 3H, CH_3), 1.35-1.49 (m, 2H, CH_2), 1.60-1.73 (m, 2H, CH_2), 2.66 (t, *J* = 7.62 Hz, 2H, CH_2), 6.58 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.15 (ddd, *J* = 8.06, 7.18, 1.17 Hz, 1H, Ar-H), 7.29 (s, 4H, Ar-H), 7.36-7.48 (m, 3H, Ar-H), 7.62 (ddd, *J* = 8.57, 7.25, 1.76 Hz, 1H, Ar-H), 7.75-7.80 (m, 1H, Ar-H), 7.88 (d, *J* = 15.53 Hz, 1H, CH=CH), 8.15 (dd, *J* = 8.20, 1.47 Hz, 1H, Ar-H), 8.87 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 11.18 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(2', 6'-Dimethyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (15)



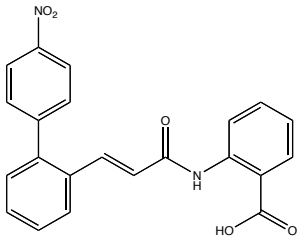
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 2,6-dimethylphenylboronic acid (25.0 mg, 0.17 mmol) according to the general procedure. The obtained clear yellow solution with white precipitate was filtered and purified by HPLC, eluting with acetonitrile (50-95%) in water. The title compound was isolated as a white solid (29.5 mg, 0.08 mmol, 48% yield).

LC-MS: R_t 2.97 mins; m/z (ESI): [M-H]- 370.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 1.95 (s, 6H, CH_3 & CH_3), 6.37 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.08-7.24 (m, 5H, Ar-H), 7.37-7.49 (m, 3H, Ar-H & CH=CH), 7.58 (ddd, *J* = 8.64, 7.33, 1.61 Hz, 1H, Ar-H), 7.80 (dd, *J* = 7.47, 1.61 Hz, 1H, Ar-H), 8.12 (dd, *J* = 7.91, 1.47 Hz, 1H, Ar-H), 8.81 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 11.03 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(4'-Nitro-biphenyl-2-yl)-acryloylamino]-benzoic acid (16)



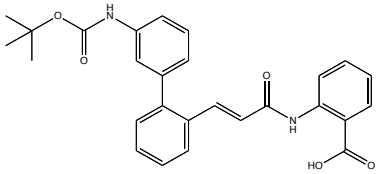
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 4-nitrophenylboronic acid (27.9 mg, 0.17 mmol) according to the general procedure. The obtained dark yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (30.1 mg, 0.08 mmol, 46% yield).

LC-MS: R_t 2.74 mins; m/z (ESI): [M+H]+ 389; [M-H]- 387.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.88 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.12-7.18 (m, 1H, Ar-H), 7.43-7.60 (m, 5H, Ar-H & CH=CH), 7.62-7.68 (m, 2H, Ar-H), 7.97-8.05 (m, 2H, Ar-H), 8.38 (m, 2H, Ar-H), 8.52 (d, *J* = 8.20 Hz, 1H, Ar-H), 11.70 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(3'-tert-Butoxycarbonylamino-biphenyl-2-yl)-acryloylamino]-benzoic acid (17)



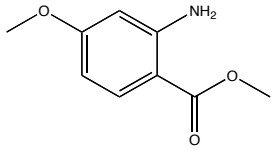
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 3-(boc-amino)phenylboronic acid (39.6 mg, 0.17 mmol) according to the general procedure. The obtained dark greenish yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (50-95%) in water. The title compound was isolated as a white solid (57.4 mg, 0.13 mmol, 75% yield).

LC-MS: R_t 2.91 mins; m/z (ESI): [M-H]- 457.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 1.60 (s, 9H, $(CH_3)_3$), 6.67 (d, *J* = 17.00 Hz, 1H, CH=CH), 7.05-7.13 (m, 3H, Ar-H), 7.35-7.46 (m, 4H, Ar-H), 7.53-7.65 (m, 3H, Ar-H & CH=CH), 7.80 (d, *J* = 7.33 Hz, 1H, Ar-H), 8.11 (d, *J* = 8.20 Hz, 1H, Ar-H), 8.79 (dd, *J* = 8.50, 1.17 Hz, 1H, Ar-H), 11.71 (br.s., 1H, NH).

Purity: >95% by HPLC.

2-Amino-4-methoxy benzoic acid methyl ester (18a)



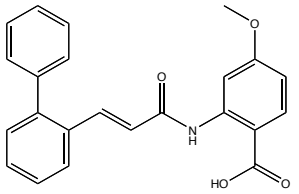
According to the general procedure, 4-methoxyanthranilic acid (60.0 mg, 0.36 mmol), dissolved in dry tetrahydrofuran (1.25 mL), was esterified with trimethylsilyl diazomethane (0.29 ml, 0.57 mmol) with dry methanol (0.14 mL) added. The clear brown solution obtained was purified by flash chromatography (hexanes:ethyl acetate 9:1). The title compound was obtained as a white solid (34.5 mg, 0.19 mmol, 53% yield).

TLC: R_f = 0.5 (hexanes:ethyl acetate 2:1).

LC-MS: R_t 1.91 mins; m/z (ESI): [M-H]- 180.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 3.77 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 6.17 (dd, *J* = 9.08, 2.64 Hz, 1H, Ar-H), 6.24 (d, *J* = 2.34 Hz, 1H, Ar-H), 7.70 (d, *J* = 8.79 Hz, 1H, Ar-H).

2-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methoxy-benzoic acid (18)



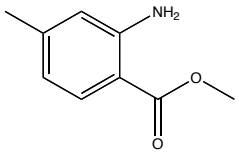
(E)-3-Biphenyl-2-yl-acrylic acid (21.5 mg, 0.10 mmol) was dissolved in dichloromethane (3 mL) and cooled on ice. Thionyl chloride (0.11 mL, 1.44 mmol) was added and the resulting solution was heated to reflux at 50ºC for 2 hours. The reaction mixture was concentrated and re-dissolved in dimethylformamide (1.5 mL). 2-Amino-4-methoxy benzoic acid methyl ester **18a** (23.3 mg, 0.10 mmol) dissolved in dimethylformamide (1 mL) was added to the reaction mixture. Pyridine (9.1 µL, 0.11 mmol) and catalytic amounts of dimethylaminopyridine were added and the resulting mixture was subjected to microwave irradiation at 130ºC for 6 hours. The reaction did not run to completion but was stopped when about 50% of title compound was formed. The obtained mixture was purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (15.0 mg, 0.04 mmol, 42% yield).

LC-MS: R_t 2.85 mins; m/z (ESI): [M+H]+ 374; [M-H]- 372.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 3.89 (s, 3H, O-CH_3), 6.55 (d, *J* = 15.53 Hz, 1H, CH=CH), 6.65 (dd, *J* = 8.94, 2.49 Hz, 1H, Ar-H), 7.33-7.49 (m, 8H, Ar-H), 7.76-7.79 (m, 1H, Ar-H), 7.82 (d, *J* = 15.53 Hz, 1H, CH=CH), 8.04 (d, *J* = 9.08 Hz, 1H, Ar-H), 8.53 (d, *J* = 2.64 Hz, 1H, Ar-H), 11.38 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-Amino-4-methyl benzoic acid methyl ester (19a)



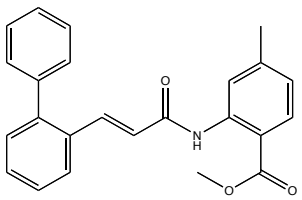
According to the general procedure, 2-amino-4-methyl benzoic acid (60.0 mg, 0.40 mmol), dissolved in dry tetrahydrofuran (1.25 mL), was esterified with trimethylsilyl diazomethane (0.32 ml, 0.64 mmol) with dry methanol (0.14 mL) added. The brown yellow solution obtained was purified by flash chromatography (hexanes:ethyl acetate 6:1). The title compound was obtained as a white crystalline solid (44.3 mg, 0.27 mmol, 68% yield).

TLC: R_f = 0.12 (hexanes:ethyl acetate 9:1).

LC-MS: R_t 1.30 mins; m/z (ESI): [M+H]+ 166; [M-H]- 164.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 2.20 (s, 3H, CH_3), 3.88 (s, 3H, O-CH_3), 7.11 (d, *J* = 7.62 Hz, 1H, Ar-H), 7.34-7.40 (m, 2H, Ar-H).

2-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methyl-benzoic acid methyl ester (19b)



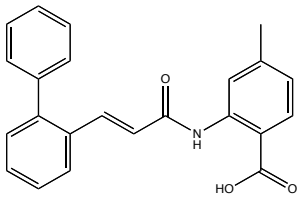
(E)-3-Biphenyl-2-yl-acrylic acid (80.0 mg, 0.36 mmol) was dissolved in dichloromethane (11 mL) and cooled on ice. Thionyl chloride (0.39 mL, 5.36 mmol) was added and the resulting solution was heated to reflux at 50ºC for 3 hours 45 minutes. The reaction mixture was concentrated and re-dissolved in dimethylformamide (5 mL). 2-Amino-4-methyl benzoic acid

methyl ester **19a** (59.0 mg, 0.36 mmol), pyridine (33.8 μL, 0.42 mmol) and catalytic amounts of dimethylaminopyridine were subsequently added and the resulting mixture was subjected to microwave irradiation at 130ºC for 7 hours. The obtained pale yellow brown mixture was purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white crystalline solid (43.8 mg, 0.12 mmol, 33% yield).

LC-MS: R_t 2.86 mins; m/z (ESI): [M+H]+ 372; [M-H]- 370.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 2.33 (s, 3H, CH_3), 3.89 (s, 3H, O-CH_3), 6.50 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.07 (s, 1H, Ar-H), 7.28 (d, *J* = 7.91 Hz, 1H, Ar-H), 7.32-7.49 (m, 8H, Ar-H), 7.70-7.81 (m, 2H, Ar-H), 7.79 (d, *J* = 15.53 Hz, 1H, CH=CH), 8.57 (br.s., 1H, NH).

2-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methyl-benzoic acid (19)



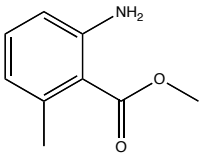
2-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methyl-benzoic acid methyl ester **19b** (32.0 mg, 0.09 mmol) was dissolved in tetrahydrofuran (2.5 mL). 4M lithium hydroxide (0.83 mL, 3.33 mmol) was added and the reaction mixture was stirred at room temperature for 72 hours. The reaction did not run to completion but was stopped when about 60% of title compound were formed. After tetrahydrofuran was removed under reduced pressure, water (1 mL) was added. The aqueous layer was acidified using 3N hydrochloric acid and extracted with ethyl acetate (3 x 5 mL). The organic layers were collected, dried over sodium sulfate and concentrated. The crude reaction mixture was purified by HPLC, eluting with acetonitrile (40-95%) in water. The title compound was isolated as a white solid (15.6 mg, 0.04 mmol, 51% yield).

LC-MS: R_t 2.51 mins; m/z (ESI): [M+H]+ 358; [M-H]- 356.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.31 (s, 3H, CH_3), 7.02 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.31-7.56 (m, 10H, Ar-H & CH=CH), 7.63 (dd, *J* = 7.62, 1.76 Hz, 1H, Ar-H), 7.81-7.85 (m, 1H, Ar-H), 8.22 (d, *J* = 1.47 Hz, 1H, Ar-H), 9.57 (br.s., 1H, NH).

Purity: >95% by HPLC.

2-Amino-6-methyl benzoic acid methyl ester (20a)



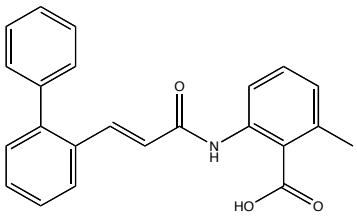
According to the general procedure, 2-amino-6-methyl benzoic acid (150.0 mg, 0.99 mmol), dissolved in dry tetrahydrofuran (3.2 mL), was esterified with trimethylsilyl diazomethane (0.79 ml, 1.59 mmol) with dry methanol (0.35 mL) added. The pale brown yellow solution obtained was purified by flash chromatography (hexanes:ethyl acetate 6:1). The title compound was obtained as a clear oil (129.9 mg, 0.79 mmol, 79% yield).

TLC: R_f = 0.12 (hexanes:ethyl acetate 8:1).

LC-MS: R_t 1.77 mins; m/z (ESI): [M+H]+ 166.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 2.36 (s, 3H, CH_3), 3.87 (s, 3H, O-CH_3), 6.48 (dd, *J* = 7.03, 1.47 Hz, 1H, Ar-H), 6.61 (dd, *J* = 8.50, 0.59 Hz, 1H, Ar-H), 7.04 (t, *J* = 7.62 Hz, 1H, Ar-H).

2-[(E)-3-Biphenyl-2-yl-acryloylamino]-6-methyl-benzoic acid (20)



(E)-3-Biphenyl-2-yl-acrylic acid (171.0 mg, 0.76 mmol) was dissolved in dichloromethane (20 mL) and cooled on ice. Thionyl chloride (0.84 mL, 11.45 mmol) was added and the resulting solution was heated to reflux at 50ºC for 5 hours. The completed reaction was concentrated and re-dissolved in dimethylformamide (2.5 mL). A solution of 2-amino-6-methyl benzoic acid methyl ester **20a** (126.0 mg, 0.76 mmol) in dimethylformamide (2.5 mL), pyridine (72.2 μL, 0.89 mmol) and catalytic amounts of dimethylaminopyridine were subsequently added and the resulting mixture was subjected to microwave irradiation at 130ºC for 6 hours. The obtained pale brown mixture was purified by HPLC, eluting with

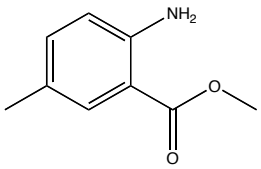
acetonitrile (40-95%) in water. The title compound was isolated as a pale yellow solid (45.6 mg, 0.13 mmol, 17% yield).

LC-MS: R_t 2.63 mins; m/z (ESI): [M+H]+ 358; [M-H]- 356.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 2.56 (s, 3H, CH_3), 6.50 (d, *J* = 15.82 Hz, 1H, CH=CH), 7.01 (d, *J* = 7.33 Hz, 1H, Ar-H), 7.31-7.47 (m, 9H, Ar-H), 7.70-7.75 (m, 1H, Ar-H), 7.78 (d, *J* = 15.53 Hz, 1H, CH=CH), 8.38 (d, *J* = 8.50 Hz, 1H, Ar-H), 10.11 (br.s., 1H, NH).

Purity: >95% by HPLC.

2-Amino-5-methyl benzoic acid methyl ester (21a)



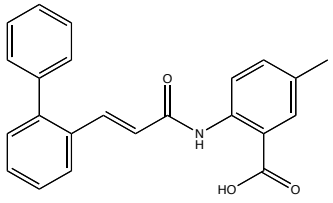
According to the general procedure, 2-amino-5-methyl benzoic acid (150.0 mg, 0.99 mmol), dissolved in dry tetrahydrofuran (3.2 mL), was esterified with trimethylsilyl diazomethane (0.79 ml, 1.59 mmol) with dry methanol (0.35 mL) added. The pale brown yellow solution obtained was purified by flash chromatography (hexanes:ethyl acetate 9:1). The title compound was obtained as a pale yellow crystalline solid (153.9 mg, 0.93 mmol, 94% yield).

TLC: R_f = 0.43 (hexanes:ethyl acetate 4:1).

LC-MS: R_t 2.05 mins; m/z (ESI): [M+H]+ 166.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 2.19 (s, 3H, CH_3), 3.84 (s, 3H, O-CH_3), 6.67 (d, *J* = 8.50 Hz, 1H, Ar-H), 7.06-7.10 (m, 1H, Ar-H), 7.58-7.60 (m, 1H, Ar-H).

2-[(E)-3-Biphenyl-2-yl-acryloylamino]-5-methyl-benzoic acid (21)



(E)-3-Biphenyl-2-yl-acrylic acid (153.4.0 mg, 0.68 mmol) was dissolved in dichloromethane (8 mL) and cooled on ice. Thionyl chloride (0.75 mL, 10.26 mmol) was added and the resulting solution was heated to reflux at 50ºC for 2 hours. The reaction mixture was

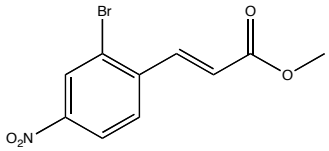
concentrated and re-dissolved in dimethylformamide (5 mL). 2-Amino-5-methyl benzoic acid methyl ester **21a** (113.0 mg, 0.68 mmol), pyridine (64.7 µL, 0.80 mmol) and catalytic amounts of dimethylaminopyridine were subsequently added and the resulting mixture was subjected to microwave irradiation at 130ºC for 5 hours 30 minutes. The obtained brown mixture was purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as an off-white solid (21.6 mg, 0.06 mmol, 9% yield).

LC-MS: R_t 2.63 mins; m/z (ESI): [M-H]- 356.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 2.33 (s, 3H, CH_3), 6.64 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.30-7.48 (m, 9H, Ar-H), 7.71 (d, *J* = 15.82 Hz, 1H, CH=CH), 7.80-7.84 (m, 1H, Ar-H), 7.91 (d, *J* = 2.34 Hz, 1H, Ar-H), 8.48 (d, *J* = 8.50 Hz, 1H, Ar-H).

Purity: >95% by HPLC.

(E)-3-(2-Bromo-4-nitro-phenyl)-acrylic acid methyl ester (22a)



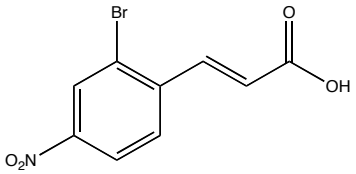
Methyl diethyl phosphonoacetate (0.33 mL, 1.83 mmol) was dissolved in anhydrous tetrahydrofuran (4 mL) and cooled to -78ºC under argon. Butyllithium (1.14 mL, 1.83 mmol) was then slowly added over 10 minutes and the resulting solution was stirred for 5 minutes. The solution was transferred to an ice bath and stirred for another 30 minutes. A solution of 2-bromo-4-nitrobenzaldehyde (300.0 mg, 1.30 mmol) in dry tetrahydrofuran (4 mL) was added to the final reaction mixture, which was stirred for 5 hours. The reaction mixture was quenched with saturated ammonium chloride (30 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic layers were dried over sodium sulfate and concentrated. The crude reaction mixture was purified by flash chromatography (hexanes:ethyl acetate 9:1). The title compound was obtained as a pale yellow solid (305.6 mg, 1.07 mmol, 82% yield).

TLC: R_f = 0.37 (hexanes:ethyl acetate 8:1).

LC-MS: R_t 2.52 mins; m/z (ESI): [M+H]+ 287.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 3.86 (s, 3H, O-CH_3), 6.51 (d, *J* = 16.12 Hz, 1H, CH=CH), 7.75 (d, *J* = 8.50 Hz, 1H, Ar-H), 8.03 (d, *J* = 16.12 Hz, 1H, CH=CH), 8.20 (ddd, *J* = 8.64, 1.17, 1.03 Hz, 1H, Ar-H), 8.50 (d, *J* = 2.34 Hz, 1H, Ar-H).

(E)-3-(2-Bromo-4-nitro-phenyl)-acrylic acid (22b)

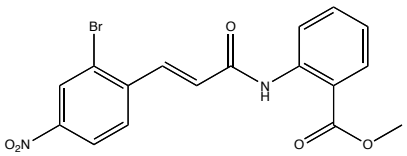


(E)-3-(2-Bromo-4-nitro-phenyl)-acrylic acid methyl ester **22a** (305.6.0 mg, 1.07 mmol) was dissolved in tetrahydrofuran (2.8 mL). 4M lithium hydroxide (0.94 mL, 3.74 mmol) was added and the reaction mixture was subjected to microwave irradiation at 65ºC for 1 hour. After tetrahydrofuran was removed under reduced pressure, water (10 mL) was added. The aqueous layer was acidified using 3N hydrochloric acid resulting in an orange precipitate. Filtration of the precipitate yielded the title compound as an orange solid (210.4 mg, 0.77 mmol, 72% yield).

LC-MS: R_t 2.05 mins; m/z (ESI): [M-H]- 271.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 6.65 (d, *J* = 15.82 Hz, 1H, CH=CH), 7.98-8.05 (m, 2H, Ar-H & CH=CH), 8.23 (dd, *J* = 2.34, 0.59 Hz, 1H, Ar-H), 8.52 (d, *J* = 2.34 Hz, 1H, Ar-H).

2-[(E)-3-(2-Bromo-4-nitro-phenyl)-acryloylamino]-benzoic acid methyl ester (22c)



(E)-3-(2-Bromo-4-nitro-phenyl)-acrylic acid **22b** (210.4 mg, 0.77 mmol) was dissolved in dichloromethane (4 mL) and cooled on ice. Thionyl chloride (0.85 mL, 11.6 mmol) was added and the resulting solution was heated to reflux at 50ºC for 2 hours 20 minutes. The reaction mixture was concentrated and re-dissolved in dichloromethane (3 mL). Methyl anthranilate (100.0 µL, 0.77 mmol), pyridine (75.0 µL, 0.93 mmol) and catalytic amounts of dimethylaminopyridine were subsequently added and the resulting mixture was stirred at room temperature for 20 hours 30 minutes. Dichloromethane (20 mL) was added to the

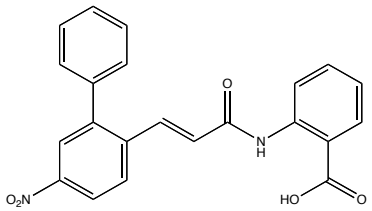
reaction mixture and the organic layer was washed with 1M hydrochloric acid (2 x 20 mL), dried over sodium sulfate and concentrated. The crude orange solid was purified by flash chromatography (eluent system of hexanes:ethyl acetate 4:1). The title compound was obtained as a bright yellow solid (189.2 mg, 0.47 mmol, 60% yield).

TLC: R_f = 0.68 (hexanes:ethyl acetate 1:1).

LC-MS: R_t 2.98 mins; m/z (ESI): [M+H]+ 406; [M-H]- 404.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 3.97 (s, 3H, O-CH_3), 6.70 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.13-7.19 (m, 1H, Ar-H), 7.62 (ddd, *J* = 8.64, 7.18, 1.76 Hz, 1H, Ar-H), 7.82 (d, *J* = 8.79 Hz, 1H, Ar-H), 8.07-8.12 (m, 1H, Ar-H), 8.11 (d, *J* = 15.82 Hz, 1H, CH=CH), 8.21 (ddd, *J* = 8.79, 2.34, 0.59 Hz, 1H, Ar-H), 8.51 (d, *J* = 2.05 Hz, 1H, Ar-H), 8.88 (dd, *J* = 8.64, 1.03 Hz, 1H, Ar-H), 11.58 (br.s., 1H, NH).

2-[(E)-3-(5-Nitro-biphenyl-2-yl)-acryloylamino]-benzoic acid (22)



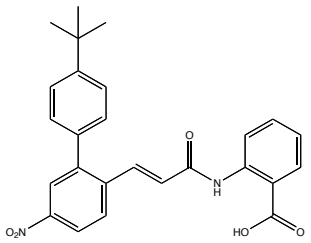
2-[(E)-3-(2-Bromo-4-nitro-phenyl)-acryloylamino]-benzoic acid methyl ester **22c** (79.3 mg, 0.20 mmol) was coupled with phenylboronic acid (23.9 mg, 0.20 mmol) following the general procedure. The obtained brown red solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (53.0 mg, 0.14 mmol, 70% yield).

LC-MS: R_t 2.83 mins; m/z (ESI): [M-H]- 387.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 6.83 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.10-7.17 (m, 1H, Ar-H), 7.36-7.41 (m, 2H, Ar-H), 7.45-7.56 (m, 4H, Ar-H), 7.70 (d, *J* = 15.53 Hz, 1H, CH=CH), 8.03 (d, *J* = 8.50 Hz, 1H, Ar-H), 8.10 (dd, *J* = 7.91, 1.76 Hz, 1H, Ar-H), 8.21 (d, *J* = 2.05 Hz, 1H, Ar-H), 8.24-8.28 (m, 1H, Ar-H), 8.62 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H).

Purity: >98% by HPLC.

2-[(E)-3-(4'-tert-Butyl-5-nitro-biphenyl-2-yl)-acryloylamino]-benzoic acid (23)



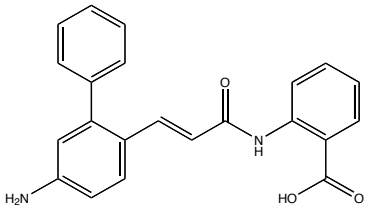
2-[(E)-3-(2-Bromo-4-nitro-phenyl)-acryloylamino]-benzoic acid methyl ester **22c** (68.4 mg, 0.17 mmol) was coupled with 4-tert-butylphenylboronic acid (30.1 mg, 0.17 mmol) following the general procedure. The obtained brown red solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (50-95%) in water. The title compound was isolated as a pale brown solid (47.4 mg, 0.11 mmol, 63% yield).

LC-MS: R_t 2.83 mins; m/z (ESI): [M+H]+ 445; [M-H]- 443.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 6.69 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.14-7.20 (m, 1H, Ar-H), 7.29-7.34 (m, 2H, Ar-H), 7.49-7.54 (m, 2H, Ar-H), 7.63 (ddd, *J* = 8.64, 7.18, 1.76 Hz, 1H, Ar-H), 7.86 (d, *J* = 9.67 Hz, 1H, CH=CH), 7.90 (d, *J* = 2.93 Hz, 1H, Ar-H), 8.14 (dd, *J* = 7.91, 1.47 Hz, 1H, Ar-H), 8.22 (dd, *J* = 8.79, 2.64 Hz, 1H, Ar-H), 8.27 (d, *J* = 2.05 Hz, 1H, Ar-H), 8.84 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 11.29 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-[(E)-3-(5-Amino-biphenyl-2-yl)-acryloylamino]-benzoic acid (24)



2-[(E)-3-(5-Nitro-biphenyl-2-yl)-acryloylamino]-benzoic acid **22** (43.3 mg, 0.11 mmol) was reduced following the general procedure. The crude reaction mixture was purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (12.8 mg, 0.04 mmol, 32% yield).

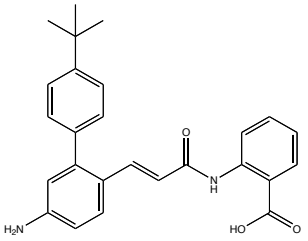
LC-MS: R_t 2.39 mins; m/z (ESI): [M+H]+ 359; [M-H]- 357.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 6.42 (d, *J* = 15.24 Hz, 1H, CH=CH), 6.63 (d, *J* = 2.34 Hz, 1H, Ar-H), 6.74 (dd, *J* = 8.79, 2.64 Hz, 1H, Ar-H), 7.07-7.14 (m, 1H, Ar-H), 7.29-7.55

(m, 6H, Ar-H & CH=CH), 7.61-7.68 (m, 2H, Ar-H), 8.08 (dd, *J* = 8.06, 1.32 Hz, 1H, Ar-H), 8.59 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H).

Purity: >98% by HPLC.

2-[(E)-3-(5-Amino-4'-tert-butyl-biphenyl-2-yl)-acryloylamino]-benzoic acid (25)



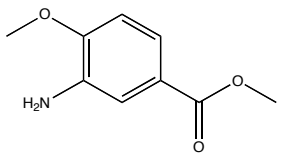
2-[(E)-3-(4'-tert-Butyl-5-nitro-biphenyl-2-yl)-acryloylamino]-benzoic acid **23** (38.4 mg, 0.09 mmol) was reduced to following the general procedure. The crude reaction mixture was purified by HPLC, eluting with acetonitrile (50-95%) in water. The title compound was isolated as a yellow solid (22.5 mg, 0.05 mmol, 63% yield).

LC-MS: R_t 2.89 mins; m/z (ESI): [M+H]+ 415; [M-H]- 413.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 1.36 (s, 9H, $(CH_3)_3$), 6.41 (d, *J* = 15.24 Hz, 1H, CH=CH), 6.64-6.72 (m, 2H, Ar-H), 7.10 (ddd, *J* = 8.28, 7.25, 1.17 Hz, 1H, Ar-H), 7.25-7.30 (m, 2H, Ar-H), 7.41-7.46 (m, 2H, Ar-H), 7.58 (ddd, *J* = 8.64, 7.33, 1.61 Hz, 1H), 7.64 (d, *J* = 8.50 Hz, 1H, Ar-H), 7.81 (d, *J* = 15.24 Hz, 1H, CH=CH), 8.11 (dd, *J* = 8.06, 1.32 Hz, 1H, Ar-H), 8.85 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 11.05 (br.s., 1H, NH).

Purity: >99% by HPLC.

3-Amino-4-methoxy benzoic acid methyl ester (26a)



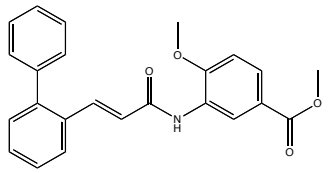
According to the general procedure, 3-amino-4-methoxy benzoic acid (160.0 mg, 0.96 mmol), dissolved in dry tetrahydrofuran (3.2 mL), was esterified with trimethylsilyl diazomethane (077 ml, 1.53 mmol) with dry methanol (0.35 mL) added. The brown solution obtained was purified by flash chromatography (hexanes:ethyl acetate 4:1). The title compound was obtained as an off-white solid (137.6 mg, 0.76 mmol, 79% yield).

TLC: R_f = 0.21 (hexanes:ethyl acetate 4:1).

LC-MS: R_t 1.12 mins; m/z (ESI): [M+H]+ 182.

^{1}H NMR (300 MHz, MeOD-*d*$_4$): δ = 3.83 (s, 3H, CO_2CH_3), 3.91 (s, 3H, O-CH_3), 6.89 (d, *J* = 7.91 Hz, 1H, Ar-H), 7.38-7.40 (m, 1H, Ar-H) 7.42 (d, *J* = 2.05 Hz, 1H, Ar-H).

3-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methoxy-benzoic acid methyl ester (26b)

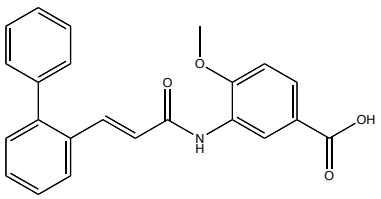


(E)-3-Biphenyl-2-yl-acrylic acid (168.0 mg, 0.75 mmol) was dissolved in dichloromethane (10 mL) and cooled on ice. Thionyl chloride (0.82 mL, 11.24 mmol) was added and the resulting solution was heated to reflux at 50ºC for 1 hour 20 minutes. The reaction mixture was concentrated and re-dissolved in dimethylformamide (2.5 mL). A solution of 3-amino-4-methoxy benzoic acid methyl ester **26a** (135.6.0 mg, 0.75 mmol) in dimethylformamide (2.5 mL), pyridine (70.9 μL, 0.88 mmol) and catalytic amounts of dimethylaminopyridine were added and the resulting mixture was subjected to microwave irradiation at 130ºC for 4 hours 30 minutes. The obtained mixture was purified by HPLC, eluting with acetonitrile (40-95%) in water. The title compound was isolated as an off-white solid (39.9 mg, 0.10 mmol, 14% yield).

LC-MS: R_t 2.94 mins; m/z (ESI): [M+H]+ 388; [M-H]- 386.

^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ = 3.87 (s, 3H, CO_2CH_3), 3.97 (s, 3H, O-CH_3), 6.54 (d, *J* = 15.53 Hz, 1H, CH=CH), 6.93 (d, *J* = 8.50 Hz, 1H, Ar-H), 7.32-7.49 (m, 8H, Ar-H & CH=CH), 7.73-7.78 (m, 1H, Ar-H), 7.80-7.82 (m, 1H, Ar-H), 7.84 (d, *J* = 2.05 Hz, 1H, Ar-H), 7.88 (br.s., 1H, NH), 9.11 (d, *J* = 2.05 Hz, 1H, Ar-H).

3-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methoxy-benzoic acid (26)



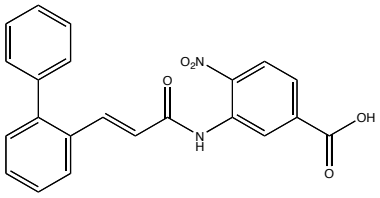
3-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-methoxy-benzoic acid methyl ester **26b** (37.9 mg, 0.10 mmol) was dissolved in tetrahydrofuran (0.74 mL). 4M lithium hydroxide (0.25 mL, 0.10 mmol) was added and the reaction mixture was subjected to microwave irradiation at 65ºC for 3 hours 30 minutes. The reaction did not go to completion but was stopped when about 80% of title compound was formed. After tetrahydrofuran was removed under reduced pressure, water (5 mL) was added. The aqueous layer was acidified using 3N hydrochloric acid and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were dried over sodium sulfate and concentrated. The crude reaction mixture was purified by HPLC, eluting with acetonitrile (40-95%) in water. The title compound was isolated as a white solid (26.7 mg, 0.07 mmol, 73% yield).

LC-MS: R_t 2.58 mins; m/z (ESI): [M+H]+ 374; [M-H]- 372.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.93 (s, 3H, O-CH_3), 7.13 (d, *J* = 8.79 Hz, 1H, Ar-H), 7.22 (d, *J* = 15.53, 1H, CH=CH), 7.33 (dd, *J* = 8.20, 1.76 Hz, 1H, Ar-H), 7.36-7.54 (m, 8H, Ar-H & CH=CH), 7.69 (dd, *J* = 8.50, 2.34 Hz, 1H, Ar-H), 7.81-7.85 (m, 1H, Ar-H), 8.72 (t, *J* = 5.27, 2.64 Hz, 1H, Ar-H), 9.45 (br.s., 1H, NH).

Purity: >99% by HPLC.

3-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-nitro-benzoic acid (27)



(E)-3-Biphenyl-2-yl-acrylic acid (300.0 mg, 1.34 mmol) was dissolved in dichloromethane (18 mL) and cooled on ice. Thionyl chloride (1.47 mL, 20.1 mmol) was added and the resulting solution was heated to reflux at 50ºC for 2 hours 35 minutes. The reaction mixture was concentrated and re-dissolved in dimethylformamide (5 mL). Methyl-3-amino-4-nitrobenzoate (262.9 mg, 1.34 mmol), pyridine (0.13 mL, 1.57 mmol) and catalytic amounts of dimethylaminopyridine were added and the resulting mixture was subjected to microwave irradiation at 130ºC for 5 hours. The obtained mixture was purified by HPLC, eluting with

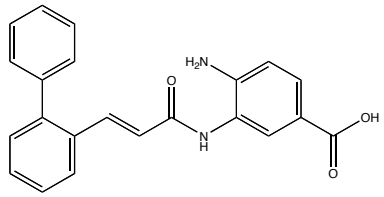
acetonitrile (50-95%) in water. The title compound was isolated as a yellow solid (262.8 mg, 0.68 mmol, 50% yield).

LC-MS: R_t 2.72 mins; m/z (ESI): [M+H]+ 389; [M-H]- 387.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 6.82 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.31-7.51 (m, 8H, Ar-H), 7.76 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.86-7.93 (m, 2H, Ar-H), 8.15 (d, *J* = 9.08 Hz, 1H, Ar-H), 8.74 (d, *J* = 1.76 Hz, 1H, Ar-H).

Purity: >97% by HPLC.

4-Amino-3-[(E)-3-biphenyl-2-yl-acryloylamino]-benzoic acid (28)



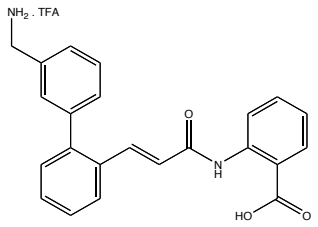
3-[(E)-3-Biphenyl-2-yl-acryloylamino]-4-nitro-benzoic acid **27** (69.0 mg, 0.18 mmol) was reduced following the general procedure. The crude reaction mixture was purified by HPLC, eluting with acetonitrile (40-95%) in water. The title compound was isolated as a white solid (21.2 mg, 0.06 mmol, 33% yield).

LC-MS: R_t 2.22 mins; m/z (ESI): [M+H]+ 359; [M-H]- 357.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.70 (br.s., 2H, NH_2), 6.72 (d, *J* = 8.50 Hz, 1H, Ar-H), 6.90 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.30-7.55 (m, 10H, Ar-H & CH=CH), 7.76-7.83 (m, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 9.45 (br.s., 1H, NH).

Purity: >97% by HPLC.

2-[(E)-3-(3'-Aminomethyl-biphenyl-2-yl)-acryloylamino]-benzoic acid-TFA salt (29)



The Boc-protecting group of 2-{(E)-3-[3'-(tert-Butoxycarbonylamino-methyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (39.2 mg, 0.08 mmol) was cleaved according to the general

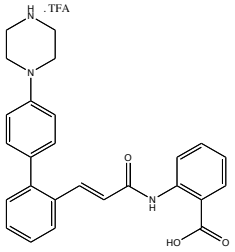
procedure. The title compound was isolated as a white solid (29.5 mg, 0.08 mmol, 96% yield).

LC-MS: R_t 2.22 mins; m/z (ESI): [M-H]- 371.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.15 (s, 2H, CH_2), 6.45 (d, *J* = 7.33 Hz, 1H, Ar-H), 6.62 (s, 1H, Ar-H), 6.82 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.04 (d, *J* = 7.62 Hz, 1H, Ar-H), 7.17 (ddd, *J* = 8.06, 7.18, 1.17 Hz, 1H, Ar-H), 7.29-7.33 (m, 1H, Ar-H), 7.38-7.50 (m, 2H, Ar-H), 7.56-7.63 (m, 2H, Ar-H), 7.64 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.94 (dd, *J* = 7.47, 1.61 Hz, 1H, Ar-H), 8.00 (dd, *J* = 7.91, 1.47 Hz, 1H, Ar-H), 8.55 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 11.28 (br.s., 1H, NH).

Purity: >98% by HPLC.

2-[(E)-3-(4'-Piperazin-1-yl-biphenyl-2-yl)-acryloylamino]-benzoic acid-TFA salt (30)



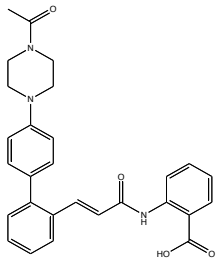
The Boc-protecting group of 4-{2'-[(E)-2-(2-Carboxy-phenylcarbamoyl)-vinyl]-biphenyl-4-yl}-piperazine-1-carboxylic acid tert-butyl ester (39.2 mg, 0.07 mmol) was cleaved according to the general procedure. The title compound was isolated as a pale brown solid (31.8 mg, 0.07 mmol, 100% yield).

LC-MS: R_t 1.77 mins; m/z (ESI): [M+H]+ 428; [M-H]- 426.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.24-3.30 (m, 4H, $(CH_2)_2$), 3.44-3.50 (m, 4H, $(CH_2)_2$), 6.80 (d, *J* = 15.53 Hz, 1H. CH=CH), 7.08-7.28 (m, 5H, Ar-H), 7.33-7.61 (m, 4H, Ar-H), 7.60 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.93 (dd, *J* = 7.62, 1.47 Hz, 1H, Ar-H), 8.00 (dd, *J* = 7.91, 1.47 Hz, 1H, Ar-H), 8.53 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 8.83 (br.s., 1H, NH), 11.48 (br.s., 1H, NH).

Purity: >96% by HPLC.

2-{(E)-3-[4'-(4-Acetyl-piperazin-1-yl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (31)



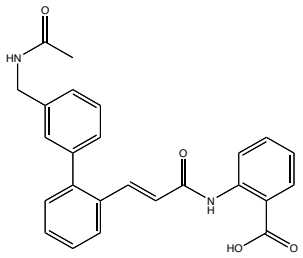
2-[(E)-3-(4'-Piperazin-1-yl-biphenyl-2-yl)-acryloylamino]-benzoic acid **30** (32.0 mg, 0.07 mmol) was dissolved in dichloromethane (3 mL). Acetic anhydride (17.7 µL, 0.19 mmol) and catalytic amounts of triethylamine were added and the resulting solution was stirred at room temperature and under argon for 50 minutes. Dichloromethane (2 mL) was added to the reaction mixture and the organic layer was washed with 1M hydrochloric acid (5 mL), dried over sodium sulfate and concentrated. The crude mixture was purified by HPLC, eluting with acetonitrile (50-95%) in water, to give the title compound as a pale yellow solid (17.4 mg, 0.04 mmol, 49% yield).

LC-MS: R_t 2.42 mins; m/z (ESI): [M+H]+ 470; [M-H]- 468.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.05 (s, 3H, $COCH_3$), 3.16-3.26 (m, 4H, $(CH_2)_2$), 3.57-3.64 (m, 4H, $(CH_2)_2$), 6.81 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.07 (d, *J* = 8.79 Hz, 2H, Ar-H), 7.12-7.19 (m, 1H, Ar-H), 7.21 (d, *J* = 8.50 Hz, 2H, Ar-H), 7.34-7.50 (m, 3H, Ar-H), 7.54-7.58 (m, 1H, Ar-H), 7.63 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.92 (dd, *J* = 7.77, 1.32 Hz, 1H, Ar-H), 8.00 (dd, *J* = 7.91, 1.76 Hz, 1H, Ar-H), 8.54 (d, *J* = 8.21 Hz, 1H, Ar-H), 11.51 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-{(E)-3-[3'-(Acetylamino-methyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (32)



2-[(E)-3-(3'-Aminomethyl-biphenyl-2-yl)-acryloylamino]-benzoic acid **29** (24.9 mg, 0.07 mmol) was dissolved in dichloromethane (2 mL). Acetic anhydride (15.8 µL, 0.18 mmol) and

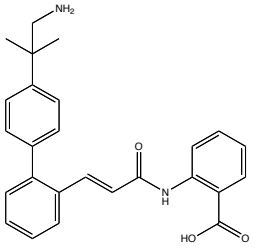
catalytic amounts of triethylamine were added and the resulting solution was stirred at room temperature and under argon for 40 minutes. Dichloromethane (2 mL) was added to the completed reaction and the organic layer was washed with 1M hydrochloric acid (4 mL), dried over sodium sulfate and concentrated. The crude mixture was purified by HPLC, eluting with acetonitrile (40-95%) in water, to give the title compound as a white solid (11.5 mg, 0.03 mmol, 42% yield).

LC-MS: R_t 2.33 mins; m/z (ESI): [M+H]+ 415; [M-H]- 413.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.06 (s, 2H, CH_2), 2.27 (s, 3H, $COCH_3$), 6.81 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.00 (dd, *J* = 7.91, 1.76 Hz, 1H, Ar-H), 7.10-7.17 (m, 1H, Ar-H), 7.28-7.35 (m, 2H, Ar-H), 7.41-7.49 (m, 3H, Ar-H), 7.50-7.60 (m, 2H, Ar-H), 7.56 (d, *J* = 15.82 Hz, 1H, CH=CH), 7.94 (dd, *J* = 7.62, 1.47 Hz, 1H, Ar-H), 7.98 (dd, *J* = 8.06, 1.61 Hz, 1H, Ar-H), 8.51 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 9.45 (br.s., 1H, NH), 11.60 (br.s., 1H, NH).

Purity: >93 % by HPLC.

2-{(E)-3-[4'-(2-Amino-1,1-dimethyl-ethyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (33)



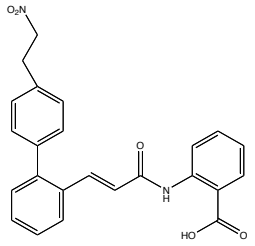
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (50.0 mg, 0.14 mmol) was coupled with the hydrochloric acid salt of 2-methyl-2-(4-bronophenyl) propylamine (31.9 mg, 0.14 mmol) following the general procedure. The obtained pale yellow solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (26.0 mg, 0.06 mmol, 45% yield).

LC-MS: R_t 1.94 mins; m/z (ESI): [M+H]+ 415; [M-H]- 413.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.44 (s, 6H, $(CH_3)_2$), 3.14 (s, 2H, CH_2), 6.40 (d, J = 16.70 Hz, 1H, CH=CH), 6.95 (td, J = 7.47, 1.17 Hz, 1H, Ar-H), 7.26-7.49 (m, 7H, Ar-H), 7.57 (d, J = 8.50 Hz, 2H, Ar-H & CH=CH), 7.84 (dd, J = 7.03, 2.05 Hz, 1H, Ar-H), 7.97 (dd, J = 7.91, 1.76 Hz, 1H, Ar-H), 8.58 (dd, J = 8.20, 0.88 Hz, 1H, Ar-H).

Purity: >99% by HPLC.

2-{(E)-3-[4'-(2-Nitro-ethyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (34)



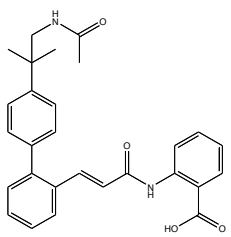
2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (70.0 mg, 0.19 mmol) was coupled with 4-(2-nitroethyl)phenylboronic acid (37.8 mg, 0.19 mmol) following the general procedure. The obtained solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (50-95%) in water. The title compound was isolated as a white solid (20.2 mg, 0.05 mmol, 25% yield).

LC-MS: R_t 2.72 mins; m/z (ESI): [M+H]+ 417; [M-H]- 416.

^{1}H NMR (300 MHz, $CDCl_3$-d): δ = 3.39 (t, J = 7.03 Hz, 2H, CH_2), 4.74 (t, J = 7.18 Hz, 2H, CH_2), 6.55 (d, J = 15.82 Hz, 1H, CH=CH), 7.12 (ddd, J = 8.06, 7.18, 1.17 Hz, 1H, Ar-H), 7.28-7.46 (m, 7H, Ar-H), 7.60 (ddd, J = 8.64, 7.18, 1.76 Hz, 1H, Ar-H), 7.71 (d, J = 15.82 Hz, 1H, CH=CH), 7.74-7.78 (m, 1H, Ar-H), 8.12 (dd, J = 7.91, 1.76 Hz, 1H, Ar-H), 8.85 (dd, J = 8.50, 0.88 Hz, 1H, Ar-H), 11.32 (br.s., 1H, NH).

Purity: >99% by HPLC.

2-{(E)-3-[4'-(2-Acetylamino-1,1-dimethyl-ethyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (35)



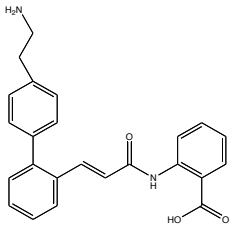
2-{(E)-3-[4'-(2-Amino-1,1-dimethyl-ethyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid **33** (22.0 mg, 0.05 mmol) was dissolved in dichloromethane (2 mL). Acetic anhydride (12.6 µL, 0.13 mmol) and catalytic amounts of triethylamine were added and the resulting solution was stirred at room temperature and under argon for 50 minutes. Dichloromethane (2 mL) was added to the completed reaction and the organic layer was washed with 1M hydrochloric acid (3 mL), dried over sodium sulfate and concentrated. The crude mixture was purified by HPLC, eluting with acetonitrile (50-95%) in water, to give the title compound as a white solid (10.2 mg, 0.02 mmol, 42% yield).

LC-MS: R_t 2.54 mins; m/z (ESI): [M+H]+ 457; [M-H]- 455.

^{1}H NMR (300 MHz, MeOD-d_4): δ = 1.38 (s, 6H, $(CH_3)_2$), 1.93 (s, 3H, $COCH_3$), 3.47 (s, 2H, CH_2), 6.70 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.16 (ddd, *J* = 8.06, 7.18, 1.17 Hz, 1H, Ar-H), 7.30-7.35 (m, 2H, Ar-H), 7.37-7.48 (m, 3H, Ar-H), 7.51-7.59 (m, 3H, Ar-H), 7.76 (d, *J* = 15.53 Hz, 1H, CH=CH), 7.85-7.89 (m, 1H, Ar-H), 8.11 (dd, *J* = 7.91, 1.76 Hz, 1H, Ar-H), 8.62 (dd, *J* = 8.35, 0.73 Hz, 1H, Ar-H).

Purity: >99% by HPLC.

2-{(E)-3-[4'-(2-Amino-ethyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid (36)



2-{(E)-3-[4'-(2-Nitro-ethyl)-biphenyl-2-yl]-acryloylamino}-benzoic acid **34** (18.9 mg, 0.05 mmol) was reduced following the general procedure. The crude reaction mixture obtained was purified by HPLC, eluting with acetonitrile (20-95%) in water. The title compound was isolated as a white solid (4.4 mg, 0.01 mmol, 25% yield).

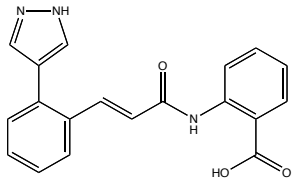
LC-MS: R_t 1.82 mins; m/z (ESI): [M+H]+ 387; [M-H]- 385.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.96 (t, *J* = 5.42 Hz, 2H, CH_2), 3.18-3.25 (m, 2H, CH_2), 6.28 (d, *J* = 16.41 Hz, 1H, CH=CH), 6.92-6.99 (m, 1H, Ar-H), 7.25-7.35 (m, 5H, Ar-H), 7.39-

7.48 (m, 4H, Ar-H & CH=CH), 7.77-7.82 (m, 1H, Ar-H), 7.97 (dd, *J* = 7.77, 1.61 Hz, 1H, Ar-H), 8.26 (br.s., 2H, NH_2), 8.53 (dd, *J* = 8.21, 0.88 Hz, 1H, Ar-H).

Purity: >97% by HPLC.

2-{(E)-3-[2-(1H-Pyrazol-4-yl)-phenyl]-acryloylamino}-benzoic acid (37)



2-[(E)-3-(2-Bromo-phenyl)-acryloylamino]-benzoic acid methyl ester (60.0 mg, 0.17 mmol) was coupled with 4,4,5,5-tetramethyl-2-[(1H)-pyrazol-4-yl]-1,3,2,-dioxaborolane (32.4 mg, 0.17 mmol) following the general procedure. The obtained solution with black precipitate was filtered and purified by HPLC, eluting with acetonitrile (30-95%) in water. The title compound was isolated as a white solid (38.4 mg, 0.12 mmol, 69% yield).

LC-MS: R_t 2.06 mins; m/z (ESI): [M+H]+ 334; [M-H]- 332.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.79 (d, *J* = 15.53 Hz, 1H, CH=CH). 7.13-7.20 (m, 1H, Ar-H), 7.33-7.49 (m, 3H, Ar-H), 7.59 (t, *J* = 7.91 Hz, 1H, Ar-H), 7.75-7.88 (m, 3H, Ar-H), 7.82 (d, *J* = 15.24 Hz, 1H, CH=CH), 8.01 (dd, *J* = 7.91, 1.47 Hz, 1H, Ar-H), 8.59 (dd, *J* = 8.50, 0.88 Hz, 1H, Ar-H), 11.58 (br. s., 1H, NH).

Purity: >96% by HPLC.

5.4. Conditions of cell-based flavivirus immunodetection (CFI) assay

A549 or BHK21 cells are trypsinized and diluted to a concentration of $2x10^5$ cells/ml in culture media (Hams F-12+2%FBS+1% penicillin/streptomycin). A 100µl of cell suspension ($2x10^4$cells) is dispensed per well into one 96-well tissue culture plate (Nunc, 96-well clear flat bottom, sterile, Nunclone Δ surface). Cells are grown overnight in culture medium at 37°C, 5% CO_2, and then infected with dengue virus at MOI=0.3 in the presence of different concentrations of test compounds for 1hr at 37°C, 5% CO_2. The virus inoculum is removed, replaced with fresh medium containing test compounds, and incubated at 37°C, 5% CO_2 for 48hrs. The cells are washed once with PBS, and fixed with cold methanol for 10min. After

washing twice with PBS, the fixed cells are blocked with PBS containing 1% FBS and 0.05% Tween-20 for 1hr at room temperature. Primary antibody (4G2) solution is subsequently added and incubated for 3hrs. The cells are washed three times with PBS followed by 1hr incubation with horseradish peroxidase (HRP)-conjugated anti-mouse IgG. After washing three times with PBS, 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution is added to each well, and the reaction is stopped by adding 0.5M sulfuric acid. The plate is read in Thermo Labsystems Multiskan Spectrum plate reader at 450 nM for viral load quantification. After measurement, the cells are washed three times with PBS, followed by incubation with propidium iodide for 5min and the reading of the plate in Tecan Safire plate reader (excitation 537nM, emission 617nM) for cell number quantification. Dose response curves are plotted from the mean absorbance versus the log of the concentration of test compounds.

References

1) Guzman M.G. & Kouri G. Dengue: an update. *Lancet, Infect. Dis.* **2**: 33-42 (2002).

2) Gubler D.J. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol*. **10**: 100-103 (2002).

3) World Health Organization. Dengue factsheet (online). <http://www.who.int/mediacentre/factsheets/fs117/en/> (2008).

4) Mukhopadhyay S., Kuhn R.J. & Rossmann M.G. A structural perspective of the *Flavivirus* life cycle. *Nat. Rev. Microbiol.* **3**: 13-22 (2005).

5) Mady B.J., Erbe D.V., Kurane I., Farger M.W. & Ennis F.A. Antibody-dependent enhancement of the dengue virus infection mediated by bipsecific antibodies against cell surface molecules other than Fc gamma receptors. *J. Immunol.* **147**: 3139-3144 (1991).

6) Leong A.S., Wong K.T., Leong T.Y., Tan P.H. & Wannakrairot P. The pathology of dengue hemorrhagic fever. *Semin. Diagn. Pathol.* **4**: 227-236 (2007).

7) Gubler D.J. & Meltzer M. The impact of dengue/dengue hemorrhagic fever on the developing world. *Adv. Virus Res.* **53**: 35-70 (1999).

8) Ray D. & Shi P.Y. Recent advances in flavivirus antiviral drug discovery and vaccine development. *Recent Patents Anti-Infect. Drug Disc.* **1**: 45-55 (2006).

9) Qi R.F., Zhang L. & Chi C.W. Biological characteristics of dengue virus and potential targets for drug design. *Acta Biochim. Biophys. Sin*. **40**: 91-101 (2008).

10) Mizuarai S., Irie H., Schmatz D.M. & Kotani H. Integrated genomic and pharmalogical approaches to identify synthetic lethal genes as cancer therapeutic targets. *Curr. Mol. Med.* **8**: 774-783 (2008).

11) Bantscheff M. et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat. Biotech.* **25**: 1035-1044 (2007).

12) Haag J.R., Pontes O. & Pikaard C.S. Metal A and metal B sites of nuclear RNA polymerases Pol IV and Pol V are required for siRNA-dependent DNA methylation and gene silencing. *PLos One.* **4**: 4110 (2009).

13) Miyaura N. & Suzuki A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **95**: 2457-2483 (1995).

14) Huang W., Guo J., Xiao Y., Zhu M., Zou G. & Tang J. Palladium–benzimidazolium salt catalyst systems for Suzuki coupling: development of a practical and highly active palladium catalyst system for coupling of aromatic halides with arylboronic acids. *Tetrahedron.* **61**: 9783-9790 (2005).

15) Wermuth C.G. *The practice of medicinal chemistry. Second edition.* (2003).