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## Synthesis and Biological Evaluation of a Series of Novel Inhibitor of Nek2/Hec1 Analogues

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### Abstract

Hec1 (High Expression in Cancer 1) is an oncogene overly expressed in many human cancers. Small molecule **INH** (Inhibitor of Nek2/Hec1) targeting the Hec1 and its regulator, Nek2, in the mitotic pathway was identified to inactivate Hec1/Nek2 function mediated by protein degradation that subsequently leads to chromosome mis-segregation and cell death. To further improve the efficacy of **INH**, a series of **INH** analogues was designed, synthesized and evaluated. Among these 33 newly-synthesized analogues, three of them, **6**, **13** and **21**, have 6-8 fold more potent cell killing activity than the previous lead compound **INH1**. Compounds **6** and **21** were chosen for analyzing the underlying action mechanism. They target directly the Hec1/Nek2 pathway and cause chromosome mis-alignment as well as cell death, a mechanism similar to that of **INH1**. This initial exploration of structural/functional relationship of **INH** may advance the progress for developing clinically applicable INH analogue.

### Introduction

Breast cancer has been one of the leading causes of death in American females for many years. Although multiple combined modality treatment strategies are available and continue to be improved, seeking for alternative therapeutics with a better therapeutic index remains a top priority of the drug development endeavors. Many of the established cancer therapeutics, represented by the microtubule poison taxane and Vinca alkaloids, inhibit cancer cell growth via direct inhibition of mitosis<sup>1, 2</sup>. Mitosis is a highly dynamic and delicate process that requires multiple layers of regulation by a variety of molecules. Several innovative agents targeting the key mitotic regulators are currently in preclinical or clinical development, including those inhibiting the spindle-associated kinesin KSP/Eg5, kinetochore-associated kinesin CENP-E, and the mitotic kinase Aurora<sup>3, 4</sup>. However, whether the downstream substrate of mitotic kinases may serve as a potential target remains to be explored.

One of the critical mitotic regulators is Hec1<sup>a</sup>, an oncogene that modulates proper spindle formation between kinetochores and centrosomes. Importantly, overexpression of Hec1 has been documented to associate with poor clinical outcomes of primary breast cancers as well

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Supporting Information Available: (A) Analytic data of compounds **4**, **8**, **10**, **12**, **15**, **17**, **19**, **25**, **36-37**. (B) Detailed procedure for preparation of compound-conjugated matrix. Both of these materials are available free of charge via <http://pubs.acs.org>.

as cases with multiple cancers<sup>5, 6</sup>. Inducible overexpression of Hec1 in a transgenic mouse model led to evident tumor formation, primarily lung adenoma and hepatocellular adenoma<sup>7</sup>. On the other hand, phosphorylation of Hec1 by Nek2 is known to be critical for its mitotic function and cell survival<sup>8, 9</sup>. We have recently identified small molecules **INH1** and **INH2** that can effectively suppress tumor cell growth in culture and in animals via directly targeting the Hec1/Nek2 mitotic pathway<sup>10</sup>. Our leads, **INH1** and **INH2**, sharing an aryl thiazolyl benzamide scaffold, came from screening a library of ~24,000 synthetic compounds through an inducible reverse yeast two-hybrid system. Although both of them inhibit the proliferation of multiple human breast cancer cell lines in culture, the potency in killing cancer cells and the water solubility remain to be improved for better efficacy. As part of a broad chemical strategy aimed toward the discovery of novel INH analogues, we designed, synthesized and evaluated a series of novel INH analogues. SAR (Structure-activity Relationship) of INH scaffold was also illustrated. **INH1** and **INH2** both have a core phenyl-thiazolyl-benzamide structures with additional groups on both sides of the thiazolyl rings (Figure 1). The main scaffold of **INH** was composed of three parts: left part (hydrophobic aryl ring), middle part (thiazolyl ring) and right part (arylamide polar part). The modification of INH analogues was mainly focused on these three parts.

## Results and Discussion

### Chemistry

Our synthesis embarked on the preparation of two intermediates **3** and **4** followed by the general synthetic procedure shown in Scheme 1. That is, treatment of bromides **1** and **2** with thiourea in refluxing EtOH afforded 4-(2,4-dimethylphenyl)thiazol-2-ylamine (**3**) and 4-(2,4,6-trimethylphenyl)thiazol-2-ylamine (**4**) in 89% and 99% yields, respectively. Acylation of amines **3** and **4** with a series of aryl acyl chloride provided the corresponding INH analogues **5-19** in medium to good yields. In addition, treatment of amines **3** and **4** with 4-morpholinecarbonyl chloride led to the compounds **20** and **21** in 63% and 39% yields, respectively. Sulphonylation of **3** with benzenesulfonyl chloride and benzylsulfonyl chloride furnished the compounds **22** and **23** in 68% and 63% yields, respectively.

To explore the influence of the hydrogen atom of amide groups on bioactivities, methylated INH analogues **24-26** were prepared (Scheme 2). Treatment of **INH1** and compound **6** with NaH / MeI / THF gave the methylated compounds **24** and **25** as the only product in 74% and 89% yields, respectively. Interestingly, subjecting **INH2** to the same condition (NaH / MeI / THF) gave two separable methylated compounds **26** and **27** in 26% and 63% yields, respectively. In our opinion, the generation of compound **27** was ascribed to the electron-donating properties of two MeO groups. That is, once **INH2** was treated with NaH in THF, the two MeO groups would result in the formation of two intermediates **28** and **29** in tautomeric equilibrium. Intermediate **29** was thought to be preferable to the intermediate **28** because the electronegative center in **29** was more remote from the two electron donating MeO groups than that in the intermediate **28**, which was consistent with the higher yield of product **27**. Furthermore, a steric contribution to the observed selectivity (higher yield of **27**) should also be taken into account as the *ortho* methoxy group would be occupying the same place as the methyl added to the amide nitrogen. Additionally, amide-protected compound **30** was prepared in 72% yield via treatment of compound **6** with NaH / BrCH<sub>2</sub>CO<sub>2</sub>Et / THF. Saponification of the ester **30** with LiOH afforded the acid **31** in 77% yield.

<sup>a</sup>**Abbreviations:** Hec1, high expression in cancer 1; Nek2, NIMA (never in mitosis gene a)-related kinase 2; INH, Inhibitor of Nek2/Hec1; RNAi, RNA interference; SAR, structure-activity relationship; DAPI, 4',6-diamidino-2-phenylindole; DMAP, 4,4-dimethylaminopyridine.

Some phenolic group-containing derivatives were also prepared (Scheme 3). Treatment of **INH2** with  $\text{BBr}_3$  (1.5 eq.) in  $\text{CH}_2\text{Cl}_2$  for 2 h smoothly gave the phenol **32** in 72% yield along with diphenol **33** in 3% yield. Further, subjecting **INH2** to  $\text{BBr}_3$  (6.5 eq.) in  $\text{CH}_2\text{Cl}_2$  for 3 hr delivered **32** in 20% yield and the diphenol **33** in 64% yield. Synthesis of compound **36** started from 2'-hydroxy-4',6'-dimethylacetophenone (**34**), which was converted to the 2-aminothiazoline **35** in three steps including methylation with  $\text{Me}_2\text{SO}_4 / \text{K}_2\text{CO}_3$ , bromination with  $\text{HBr} / \text{BuOOH}$  and final condensation of the resultant  $\alpha$ -bromoacetone with thiourea. Benzoylation of amine **35** smoothly gave the compound **36** in 93% yield. Exposure of compound **36** to  $\text{BBr}_3 / \text{CH}_2\text{Cl}_2$  at room temperature provided the phenol analogue **37** in 90% yield.

Substitution of an oxazolyl ring for the thiazolyl ring in **INH1** was also accomplished (Scheme 4). Reaction of bromide **1** with urea in refluxing EtOH gave the corresponding 2-aminooxazole **38**, which was benzoylated to afford the 2-aminooxazolyl derivative **39**. In addition, replacement of amide group in **INH1** with thioamide group was performed through treatment with **INH1** with Lawesson's reagent in refluxing toluene and the desired thioamide derivative **40** was obtained in 79% yield.

### SAR analysis of the INH analogues

Synthesized analogues **3-27**, **30-33**, **36-37** and **39-40** were tested for their killing activities on human breast cancer cell lines MDA-MB231 and MDA-MB468, human cervical cancer line HeLa and human erythromyeloblastoid leukemia cell line K562. The results are outlined in Table 1, which are the basis of the current SAR analysis. Upon removal of the benzoyl groups from **INH1** and **INH2**, the resultant thiazolyl amine **3** and **4** did not exhibit any anti-proliferative activity even at  $> 40 \mu\text{M}$ , indicating that benzoyl moiety is indispensable. Remarkably, the hybrid compound **5** containing the left moiety of **INH1** and the right moiety of **INH2** exhibited very low anti-proliferation activities against all the tested cell lines. To our delight, the other hybrid compound **6**, containing the right moiety of **INH2** and the left moiety of **INH1**, exhibited significantly improved anti-proliferation activities compared with our leads **INH1** and **2**. Additionally, deletion of the *para*-methoxyl group in **INH2** resulted in the dramatic loss of bioactivity (**7**) and replacement of the methoxyl group in **7** with trifluoromethyl group would efficiently improve the bioactivity by approximately 1.5-2 fold (**8**). Based on the above results and in light of the well-known strong electron-withdrawing properties of trifluoromethyl group that renders neighboring group polar, we proposed that 2,4,6-trimethyl phenyl is preferred to 2,4-dimethyl phenyl, probably due to less rotational flexibility, and that introduction of a more polar group on the right moiety of scaffold seems to improve the bioactivity.

A series of analogues **9-21** containing different polar groups on the right part of scaffold was prepared and the biological activities were evaluated. As expected, most analogues exhibited better cell killing activity than the original leads. For most cases, 2,4,6-trimethyl phenyl group was more active than 2,4-dimethyl phenyl group. Apparently, isonicotinoyl group (**13**) was preferred to pyridine-2-carboxyl group (**9**, **10**) and nicotinoyl group (**11**, **12**). Substitution of pyrazinyl group for isonicotinoyl group led to a considerable reduction in bioactivity (**13** vs **14**) although compound **15** still exhibited good bioactivity. The bioactivity variation between **16** and **17**, which contain 1-methyl-1H-imidazolyl groups, further consolidated our conclusion that 2,4,6-trimethyl phenyl group was preferred to 2,4-dimethyl phenyl group. Introduction of 1,3-thiazolyl groups did not change the bioactivities significantly (**18**, **19**). Notably, the replacement of phenyl group in lead **INH1** with morpholinyl group efficiently improved the activity. Among the analogues made, **6**, **13** and **21** displayed the strongest activity, the  $\text{IC}_{50}$  values of which were improved by 6-8 fold in comparison to **INH1**. The *para* position of the hetero atom (*N*, *O*) on the six-membered ring of compounds **13** and **21** clearly provides

advantages over the lead **INH1** and the pyridine-2-carboxyl derivatives **9-10** (with hetero atom *N* at ortho-position), suggesting possible existence of an extended hydrophilic groove or pocket in the target binding site.

Replacement of amide moiety in **INH1** with sulfonyl amide reduced the activity significantly (**22-23**,  $IC_{50} > 40 \mu M$ ). Upon methylation of amide groups of **INH1** or **2** and compound **6**, the resultant derivatives **24-26** showed weakened bioactivities against all the tested cell lines, indicating that methylation of the amide group negatively affects compound activity. Although compound **30** derived from ethoxycarbonylmethylating compound **6** still displayed weak activities, its saponified product **31** is almost inactive ( $IC_{50} > 40 \mu M$ ). Demethylated derivative **33** of **INH2** showed very weak activity, but monophenol compound **32** still exhibited slightly weak bioactivity compared with **INH2**, which indicated that phenol group on the para-position was not preferred. Interestingly, compounds **36-37**, derived from substitution of methoxyl group and hydroxyl group for C6 methyl group in **6**, were also found to be active although bioactivity decreased significantly. Additionally, we also found that conversion of thiazolyl ring in **INH1** to oxazolyl ring (**39**) or transformation of amide group in **INH1** to thioamide group (**40**) also made the bioactivity decrease, which demonstrated that thiazolyl ring and amide group were important for the cytotoxicity.

### Target validation of INH analogues

Next, target validation was performed by using an affinity pulldown assay described previously<sup>10</sup>. Because compounds **6**, **13** and **21** showed most improved cell killing activities with similar  $IC_{50}$  values in the SAR analysis, they were chosen for target validation by using compound **22** as a negative control. More specifically, **6** was used as a positive compound and **22** as a negative control in the affinity pulldown assay. In principle, compounds **6** and **22** were individually conjugated to an affi-gel matrix through a multistep synthetic route (Supplemental information) (Figure 2A)<sup>10</sup>. HeLa cell extract was prepared and subjected to affinity pulldown experiments with control or compound-conjugated matrices. Shown in Figure 2B, **6**-conjugated matrix, but not **22**-conjugated or unconjugated matrix, selectively co-precipitated with cellular Hec1. In contrast, none of them co-precipitated with cellular Nek2. The results suggest that compound **6** binds to cellular Hec1, similar to the lead, **INH1**.

A hallmark mechanism of action by **INH1** is to trigger dramatic reduction of Nek2 protein level<sup>10</sup>. To determine whether INH analogues retain such bioactivity in cells, both time and dosage-dependent kinetic studies were carried out in HeLa cells. The protein levels of Hec1 and Nek2 were examined by Western blot. In a doses-dependent study, Nek2 was found to be undetectable on treating cells with **INH1** ( $25 \mu M$ ), or lower dosages of **6** or **21** (both at  $6.25 \mu M$ ), but not by the negative compound **22** (up to  $25 \mu M$ ) (Figure 2C). For the time-dependent study, cells were treated with  $6.25 \mu M$  of compound **6** or **21**. Notably, the Nek2 level was reduced by ~50% at 8-11 h after treatment with **6** or **21**, which was comparable to **INH1** treatment at  $25 \mu M$  with a similar period of time (Figure 2D). In contrast, **22** showed no effect on Nek2 level at any time point tested. In addition, **6** and **21** seemed to trigger a slight Hec1 decrease over time, unlike the treatment with **INH1**, though the significance of partial Hec1 reduction is not clear at present. These results indicate that **6** and **21** are more efficient in targeting the Hec1/Nek2 complex for degradation.

### Compound 6 and 21 triggered mitotic abnormalities

The Hec1/Nek2 complex functions primarily during G2 and M phases. Perturbation of the Hec1 or Nek2 function by antagonists (RNAi or antibody) leads to mitotic abnormalities represented by spindle configuration changes and chromosome misalignment, which contribute to mitotic catastrophe<sup>11-18</sup>. Treating cells with **INH1** similarly led to mitotic abnormalities, albeit to a lesser extent<sup>10</sup>. To test whether compound **6** and **21** can elicit similar

mitotic phenotypes via inhibiting Hec1/Nek2, cells were treated with **6**, **21** or the negative compound **22**. In comparison to the mock or **22**-treated cells, **6** or **21** treated cells exhibited increased mitotic population with multipolar spindle configurations, presumably because of defective Hec1/Nek2 functionality (Figure 3A). Consistently, an increased rate of chromosome misalignment was detected upon treatment with **6** or **21** of HeLa cells expressing the chromosome marker protein H2B-GFP (Figure 3B). At the dosage of 6.25  $\mu$ M, **6** and **21** elicited mitotic abnormalities to a similar extent as **INH1** treatment at 25  $\mu$ M, further confirming the improved efficacy of compound **6** and **21**.

### Treatment of cells with compound **6** and **21** induced apoptosis

Mitotic abnormalities often lead to mitotic arrest, often followed by mitotic slippage and consequent mitotic catastrophe (i.e., cell death due to abnormal mitosis). Lead compound **INH1** is known to trigger mitotic catastrophe. To examine whether compound **6** and **21** may trigger mitotic catastrophe, a time lapse experiment was performed to monitor the cell morphological changes and potential apoptosis. As expected, **6** or **21** treated cells showed progressive morphological changes characteristic of dying cells (e.g., membrane bubbling), which is further confirmed by cell cycle profiling with FACS analysis (Figure 4A and 4B). For instance, approximately 20% of compound **6** or **22** treated cells were apoptotic 72 hrs after treatment. The above results are consistent with the mitosis-targeting activity of compound **6** and **21** with improved efficacy.

## Conclusion

This study has identified several INH analogues that show a 6-8 folds increase in cellular killing activity by targeting Hec1/Nek2 pathway. The SAR study identified a few key structural features that may serve as a guideline for further improvement of the current compounds using medicinal chemical approaches. In essence, the presence of a hydrophobic ring with less rotational freedom, such as 2,4,6-trimethyl phenyl, is preferred at the left part of the scaffold (Figure 1). Introducing hetero atoms (N, O) or a polar group to the para positions of the six-membered ring on the right side of the scaffold seems to dramatically improve both solubility and bioactivity. Finally, the integrity of the thiazolyl ring and the amide bond in the middle of the scaffold is important for maintaining compound activity.

Two of the improved versions, compound **6** and **21**, are capable of targeting the cellular Hec1/Nek2 complex, triggering mitotic abnormalities and cell death at a much lower dosage than the lead **INH1**. The structural features identified in this study may serve as a foundation for designing, synthesis and evaluation of the next generation of **INH** derivatives with further refinement for potential clinical applications.

## Experiment Section

### Cell lines and antibodies

Human breast cancer cell lines MDA-MB231 and MDA-MB468, and the cervical adenocarcinoma line HeLa were maintained in DMEM medium (Invitrogen) supplemented with 10% FBS. Antibody sources were: mouse anti-Hec1 clone 9G3 and mouse anti-p84, Rabbit anti-Nek2 polyclonal antibodies (Santa Cruz Biotech.), mouse anti-alpha-tubulin and rabbit anti-gamma tubulin (Sigma Aldrich), and secondary antibodies conjugated with Alexa dyes (Invitrogen).

### Cell killing assay

Standard XTT assays with a four-day drug treatment procedure were performed to measure the dose-dependent cytotoxicity of INH analogs in cultured cells. Triplicate sets were measured



and compiled for final data presentation. The assay was performed by using a commercial kit (Roche Scientific) following the instructions. In principle, cells were plated on 96-well dishes one day before the drug treatment, followed by drug treatment on day 2 and XTT assay on day 5 after drug addition. The absorption at 595 nm was measured with a plate reader and converted to cell survival percentages in comparison to mock treated groups.

### Microscopy and FACS analysis

Immunostaining, image processing, and FACS assays were done as detailed previously<sup>19</sup>.

### Chemistry

All reagents were used as received from commercial sources, unless specified otherwise, or prepared as described in the literature. Reactions requiring anhydrous conditions were performed in vacuum heat-dried glassware under nitrogen atmosphere. Reaction mixtures were stirred magnetically. DMF, dichloromethane and pyridine were distilled from CaH<sub>2</sub>. <sup>1</sup>H NMR spectra were recorded at either 400 MHz or 500 MHz. <sup>13</sup>C NMR spectra were recorded at either 125 MHz or 100 MHz. <sup>19</sup>F NMR spectra were recorded at 376 MHz with FCCl<sub>3</sub> as external standard and low field is positive. Chemical shifts ( $\delta$ ) are reported in ppm, and coupling constants ( $J$ ) are in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

#### 4-(2,4-Dimethyl-phenyl)thiazol-2-ylamine (3)

The mixture of compound **1** (1.28 g, 5.63 mmol) and thiourea (450 mg, 5.91 mmol) in anhydrous EtOH (20 mL) was heated to reflux for 30 min. After that, the solvent was removed in vacuo and the saturated aqueous NaHCO<sub>3</sub> was added to make the mixture basic (pH = 8-9). Then, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  30 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of all the solvent, the residue was purified by silica gel chromatography (Hexane / EtOAc = 2 : 1) to afford product **3** (1.03 g, 89%) as a solid. mp 84.5-85.5 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.43 (d,  $J$  = 8.0 Hz, 1H), 7.06-7.03 (m, 2H), 6.42 (s, 1H), 5.29 (br, 2H), 2.42 (s, 3H), 2.35 (s, 3H); MS (ESI)  $m/z$  205 (M + H<sup>+</sup>).

#### N-[4-(2,4-Dimethylphenyl)thiazol-2-yl]-2,4-dimethoxybenzamide (5)

The solution of compound **3** (82 mg, 0.40 mmol), DMAP (55 mg, 0.44 mmol) and 2, 4-dimethoxy benzoyl chloride (83 mg, 0.42 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was stirred for 1 h. After that, the mixture was directly subjected to purification by silica gel chromatography (hexane / EtOH = 3 : 1) to give compound **5** (98 mg, 67%) as a solid. mp 159-160 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.99 (s, 1H), 8.30 (d,  $J$  = 8.8 Hz, 1H), 7.49 (d,  $J$  = 7.6 Hz, 1H), 7.09-7.06 (m, 2H), 6.88 (s, 1H), 6.69 (dd,  $J$  = 8.8, 2.4 Hz, 1H), 6.56 (s,  $J$  = 2.4 Hz, 1H), 4.07 (s, 3H), 3.90 (s, 3H), 2.43 (s, 3H), 2.36 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  164.9, 162.7, 159.4, 157.3, 150.4, 137.9, 136.1, 134.7, 132.3, 131.7, 129.8, 126.8, 112.4, 110.6, 106.2, 98.9, 56.5, 55.9, 21.3, 21.2; MS (ESI)  $m/z$  369 (M + H<sup>+</sup>), 391 (M + Na<sup>+</sup>); HRMS Calcd for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>SNa (M + Na<sup>+</sup>), 391.1092 Found: 391.1095.

#### N-[4-(2,4,6-Trimethylphenyl)thiazol-2-yl]benzamide (6)

The solution of compound **4** (105 mg, 0.48 mmol), Et<sub>3</sub>N (0.30 mL, 2.15 mmol) and BzCl (75 mg, 0.53 mmol) in dioxane (5 mL) was heated to reflux for 2.5 h. After that, the mixture was cooled to room temperature and all the solvent was removed in vacuo to afford a residue, which was extracted with EtOAc (3  $\times$  30 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of all the solvent in vacuo resulted in a residue, which was purified with silica gel chromatography (Hexane / EtOAc = 15 : 1) to give compound **6** (48 mg, 31%) as a white solid. mp 192-193 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  11.63 (s, 1H), 7.52-7.73 (m, 2H), 7.51 (tt,  $J$  = 7.6, 1.2 Hz, 1H), 7.41-7.37 (m, 2H), 6.74 (s, 1H), 6.62 (s, 2H),

2.18 (s, 3H), 1.93 (s, 6H);  $^{13}\text{C}$  NMR (100.5 MHz,  $\text{CDCl}_3$ )  $\delta$  165.3, 159.1, 148.8, 137.7, 137.0, 132.6, 132.0, 131.5, 128.6, 128.4, 127.7, 111.6, 21.2, 20.5; MS (ESI)  $m/z$  323 ( $\text{M} + \text{H}^+$ ), 345 ( $\text{M} + \text{Na}^+$ ); 645 ( $2\text{M} + \text{H}^+$ ), 667 ( $\text{M} + 2\text{Na}^+$ ); HRMS Calcd for  $\text{C}_{19}\text{H}_{19}\text{N}_2\text{OS}$  ( $\text{M} + \text{H}^+$ ), 323.1218 Found: 323.1212.

### 2-Methoxy-N-[4-(2,4,6-trimethylphenyl)thiazol-2-yl]benzamide (7)

The solution of amine **4** (120 mg, 0.55 mmol) and DMAP (110 mg, 0.90 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added a solution of 2-methoxyl benzoyl chloride (120 mg, 0.70 mmol) at 0 °C. The resultant mixture was warmed to room temperature and stirred for 20min. After that,  $\text{H}_2\text{O}$  (20 mL) was added to quench the reaction and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 30$  mL). The combined organic phases were dried with anhydrous  $\text{Na}_2\text{SO}_4$ . Removal of all the solvent in vacuo resulted in a residue, which was purified with silica gel chromatography (Hexane / EtOAc = 4 : 1) to give compound **7** (150 mg, 77%) as a white solid. mp 218.5-219.5 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  11.11 (s, 1H), 8.34 (dd,  $J = 7.6, 1.2$  Hz, 1H), 7.55 (td,  $J = 7.6, 1.6$  Hz, 1H), 7.16 (t,  $J = 7.6$  Hz, 1H), 7.05 (d,  $J = 8.4$  Hz, 1H), 6.92 (s, 2H), 6.75 (s, 1H), 4.05 (s, 3H), 2.31 (s, 3H), 2.12 (s, 6H);  $^{13}\text{C}$  NMR (100.5 MHz,  $\text{CDCl}_3$ )  $\delta$  162.5, 157.7, 157.3, 149.1, 137.7, 137.3, 134.3, 132.7, 132.3, 128.2, 121.7, 119.2, 111.5, 111.1, 56.2, 21.1, 20.3; MS (ESI)  $m/z$  353 ( $\text{M} + \text{H}^+$ ), 375 ( $\text{M} + \text{Na}^+$ ); 705 ( $2\text{M} + \text{H}^+$ ), 727 ( $\text{M} + 2\text{Na}^+$ ); HRMS Calcd for  $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_2\text{SNa}$  ( $\text{M} + \text{Na}^+$ ), 375.1143 Found: 375.1139.

### Pyridine-2-carboxylic acid [4-(2,4-dimethylphenyl)thiazol-2-yl]amide (9)

To a 0 °C solution of amine **3** (90 mg, 0.44 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) was added DMAP (120 mg, 0.98 mmol) followed by picolinoyl chloride hydrochloride (80 mg, 0.45 mmol). Then, the mixture was stirred at room temperature for 4h. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **9** (118 mg, 87%) as a solid. mp 212-213 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  11.22 (br, 1H), 8.65 (d,  $J = 4.0$  Hz, 1H), 8.31 (d,  $J = 8.0$  Hz, 1H), 7.93 (t,  $J = 7.6$  Hz, 1H), 7.53 (d,  $J = 7.2$  Hz, 2H), 7.10-7.07 (m, 2H), 6.97 (s, 1H), 2.46 (s, 3H), 2.37 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  162.1, 156.3, 150.8, 148.7, 148.0, 137.9, 136.0, 131.9, 131.8, 129.7, 127.4, 126.8, 122.9, 110.8, 21.3, 21.3; MS (ESI)  $m/z$  310 ( $\text{M} + \text{H}^+$ ), 332 ( $\text{M} + \text{Na}^+$ ); HRMS Calcd for  $\text{C}_{17}\text{H}_{15}\text{N}_3\text{OSNa}$  ( $\text{M} + \text{Na}^+$ ), 332.0833 Found: 332.0825.

### N-[4-(2,4-Dimethylphenyl)thiazol-2-yl]nicotinamide (11)

To a 0 °C solution of amine **3** (97 mg, 0.47 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added DMAP (120 mg, 0.98 mmol) followed by nicotinoyl chloride hydrochloride (108 mg, 0.60 mmol). Then, the mixture was stirred at room temperature for 20min. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **11** (93 mg, 64%) as a solid. mp 210-211 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  12.55 (br, 1H), 8.64 (s, 1H), 8.61 (s, 1H), 7.93 (d,  $J = 8.0$  Hz, 1H), 7.20-7.15 (m, 2H), 6.92 (s, 1H), 6.82-6.79 (m, 2H), 2.23 (s, 6H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  164.3, 159.3, 152.9, 148.9, 138.2, 135.7, 135.2, 131.6, 130.9, 129.6, 128.2, 126.7, 123.3, 111.0, 21.2, 20.7; MS (ESI)  $m/z$  310 ( $\text{M} + \text{H}^+$ ), 332 ( $\text{M} + \text{Na}^+$ ); HRMS Calcd for  $\text{C}_{17}\text{H}_{15}\text{N}_3\text{OSNa}$  ( $\text{M} + \text{Na}^+$ ), 332.0833 Found: 332.0835.

### N-[4-(2,4-Dimethylphenyl)thiazol-2-yl]isonicotinamide (13)

To a 0 °C solution of amine **3** (75 mg, 0.37 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added DMAP (90 mg, 0.74 mmol) followed by isonicotinoyl chloride hydrochloride (85 mg, 0.48 mmol). Then, the mixture was stirred at room temperature for 20min. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **13** (101 mg, 88%) as a solid. mp 148-149 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  12.96 (br, 1H), 8.53 (d,  $J = 4.5$  Hz, 2H), 7.38 (d,  $J = 4.5$  Hz, 2H), 7.08 (d,  $J = 8.0$  Hz, 1H), 6.94 (s, 1H), 6.79-6.76 (m, 2H), 2.21 (s, 6H);  $^{13}\text{C}$  NMR (100.5 MHz,  $\text{CDCl}_3$ )  $\delta$  164.4, 159.2, 150.3, 150.1, 139.0, 138.2, 135.5,



131.7, 130.8, 129.5, 126.8, 121.1, 111.2, 21.1, 20.6; MS (ESI)  $m/z$  310 ( $M + H^+$ ), 332 ( $M + Na^+$ ); HRMS Calcd for  $C_{17}H_{15}N_3OSNa$  ( $M + Na^+$ ), 332.0833 Found: 332.0826.

#### Pyrazine-2-carboxylic acid [4-(2,4-dimethylphenyl)thiazol-2-yl]amide (14)

To a 0 °C solution of amine **3** (103 mg, 0.51 mmol) in  $CH_2Cl_2$  (2 mL) was added DMAP (75 mg, 0.61 mmol) followed by pyrazine-2-carbonyl chloride (87 mg, 0.61 mmol). Then, the mixture was stirred at room temperature for 30min. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **14** (122 mg, 77%) as a solid. mp 168.5-169.5 °C;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  11.05 (br, 1H), 9.51 (s, 1H), 8.85 (d,  $J$  = 2.0 Hz, 1H), 8.61 (t,  $J$  = 1.5 Hz, 1H), 7.48 (d,  $J$  = 8.0 Hz, 1H), 7.07-7.05 (m, 2H), 6.99 (s, 1H), 2.44 (s, 3H), 2.35 (s, 3H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  160.9, 155.9, 151.0, 148.5, 145.0, 143.2, 142.9, 138.1, 136.0, 131.8, 131.7, 129.7, 126.8, 111.2, 21.3, 21.2; MS (ESI)  $m/z$  311 ( $M + H^+$ ), 333 ( $M + Na^+$ ); HRMS Calcd for  $C_{16}H_{14}N_4OSNa$  ( $M + Na^+$ ), 333.0786 Found: 333.0781.

#### 3-Methyl-3H-imidazole-4-carboxylic acid [4-(2,4-dimethylphenyl)thiazol-2-yl]amide (16)

To a 0 °C solution of amine **3** (85 mg, 0.42 mmol) in  $CH_2Cl_2$  (2 mL) was added DMAP (118 mg, 0.97 mmol) followed by 1-methyl-1H-imidazole-5-carbonyl chloride hydrochloride (180 mg, 0.99 mmol). Then, the mixture was stirred at room temperature for 2h. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **16** (49 mg, 37%) as a solid. mp 250 °C (decomposed);  $^1H$  NMR (500 MHz,  $DMSO-D_6$ )  $\delta$  12.55 (br, 1H), 8.11 (s, 1H), 7.92 (s, 1H), 7.53 (d,  $J$  = 8.0 Hz, 1H), 7.22 (s, 1H), 7.10 (s, 1H), 7.06 (d,  $J$  = 7.5 Hz, 1H), 3.91 (s, 3H), 2.41 (s, 3H), 2.30 (s, 3H);  $^{13}C$  NMR (125 MHz,  $DMSO-D_6$ )  $\delta$  157.9, 156.9, 149.1, 143.6, 136.9, 135.1, 131.7, 131.4, 129.4, 126.4, 124.0, 110.5, 34.0, 21.0, 20.7; MS (ESI)  $m/z$  313 ( $M + H^+$ ), 335 ( $M + Na^+$ ); HRMS Calcd for  $C_{16}H_{17}N_4OS$  ( $M + H^+$ ), 313.1123 Found: 313.1131.

#### Thiazole-2-carboxylic acid [4-(2,4-dimethylphenyl)thiazol-2-yl]amide (18)

To a 0 °C solution of amine **3** (86 mg, 0.42 mmol) in  $CH_2Cl_2$  (2 mL) was added DMAP (62 mg, 0.51 mmol) followed by 1, 3-thiazole-2-carbonyl chloride (75 mg, 0.51 mmol). Then, the mixture was stirred at room temperature for 15min. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **18** (100 mg, 76%) as a solid. mp 146.5-147.5 °C;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  10.60 (br, 1H), 7.98 (d,  $J$  = 2.5 Hz, 1H), 7.71 (d,  $J$  = 3.0 Hz, 1H), 7.48 (d,  $J$  = 7.5 Hz, 1H), 7.08-7.06 (m, 2H), 6.98 (s, 1H), 2.44 (s, 3H), 2.36 (s, 3H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  161.1, 157.1, 155.6, 151.0, 144.4, 138.0, 136.0, 131.8, 131.7, 129.7, 126.8, 126.3, 111.2, 21.3, 21.2; MS (ESI)  $m/z$  316 ( $M + H^+$ ), 338 ( $M + Na^+$ ); HRMS Calcd for  $C_{15}H_{13}N_3OS_2Na$  ( $M + Na^+$ ), 338.0398 Found: 338.0391.

#### Morpholine-4-carboxylic acid [4-(2,4-dimethylphenyl)thiazol-2-yl]amide (20)

To a 0 °C solution of amine **3** (94 mg, 0.46 mmol) in  $CH_2Cl_2$  (2 mL) was added DMAP (281 mg, 2.30 mmol) followed by 4-morpholinecarbonyl chloride (117 mg, 0.78 mmol). Then, the mixture was stirred at room temperature for 3 days. After that, the whole mixture was subjected to purification by silica gel chromatography to give compound **20** (92 mg, 63%) as a solid. mp 168-169 °C;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  10.41 (br, 1H), 7.34 (d,  $J$  = 8.0 Hz, 1H), 7.07 (s, 1H), 7.04 (d,  $J$  = 7.5 Hz, 1H), 6.80 (s, 1H), 3.43 (t,  $J$  = 4.5 Hz, 4H), 3.21 (t,  $J$  = 4.0 Hz, 4H), 2.34 (s, 3H), 2.33 (s, 3H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  161.4, 154.2, 149.1, 138.1, 136.0, 132.0, 131.7, 129.6, 126.9, 110.1, 66.4, 44.1, 21.3, 20.9; MS (ESI)  $m/z$  318 ( $M + H^+$ ), 340 ( $M + Na^+$ ); HRMS Calcd for  $C_{16}H_{19}N_3O_2SNa$  ( $M + Na^+$ ), 340.1096 Found: 340.1092.

**Morpholine-4-carboxylic acid [4-(2,4,6-trimethylphenyl)thiazol-2-yl]amide (21)**

To a solution of amine **4** (112 mg, 0.51 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was added DMAP (69 mg, 0.56 mmol). The resultant solution was cooled to 0 °C and then, a solution of 4-morpholinecarbonyl chloride (80 mg, 0.54 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was added dropwise. The mixture was warmed up to room temperature and stirred for 1h. After that, DMAP (150 mg, 1.23 mmol) and 4-morpholinecarbonyl chloride (0.67 mmol) were added. The whole mixture was further stirred for 7h. H<sub>2</sub>O (20 mL) was added to quench the reaction and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL). The combined organic phases were washed with brine and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of all the solvent, the residue was purified by silica gel chromatography (Hexan / EtOAc = 3 / 1) to give compound **21** (66 mg, 39%) as a white solid. mp 183-184 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.62 (s, 1H), 6.91 (s, 2H), 6.60 (s, 1H), 3.53 (s, 4H), 3.21 (s, 4H), 2.32 (s, 3H), 2.01 (s, 6H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 162.41, 154.59, 146.50, 138.08, 137.39, 131.47, 128.60, 110.77, 66.54, 44.00, 21.24, 20.37; MS (ESI) *m/z* 332 (M + H<sup>+</sup>), 354 (M + Na<sup>+</sup>), 662 (2M + H<sup>+</sup>), 684 (M + 2Na<sup>+</sup>); HRMS Calcd for C<sub>17</sub>H<sub>22</sub>N<sub>3</sub>O<sub>2</sub>S (M + H<sup>+</sup>), 332.1433 Found: 332.1429.

**N-[4-(2,4-Dimethylphenyl)thiazol-2-yl]benzenesulfonamide (22)**

To a 0 °C solution of amine **3** (99 mg, 0.485 mmol) in pyridine (3 mL) was added DMAP (20 mg, 0.16 mmol) followed by PhSO<sub>2</sub>Cl (95 mg, 0.53 mmol). Then, the mixture was stirred at room temperature overnight. After that, the mixture was poured into a solution of aqueous 1N HCl (50 mL). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 30 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of all the solvent in vacuo resulted in a residue, which was purified with silica gel chromatography (Hexane / EtOAc = 2 : 1) to give compound **22** (113 mg, 68%) as a solid. mp 188-189 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.93 (br, 1H), 7.93 (d, *J* = 7.6 Hz, 2H), 7.55-7.43 (m, 3H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.09-7.04 (m, 2H), 6.26 (s, 1H), 2.34, 2.33 (2s, 6H); <sup>13</sup>C NMR (100.5 MHz, CDCl<sub>3</sub>) δ 168.9, 142.1, 140.3, 136.7, 136.3, 132.4, 132.2, 129.2, 128.9, 127.4, 126.7, 125.9, 103.9, 21.4, 20.6; MS (ESI) *m/z* 345 (M + H<sup>+</sup>), 367 (M + Na<sup>+</sup>); HRMS Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub>Na (M + Na<sup>+</sup>), 367.0551 Found: 367.0551.

**N-[4-(2,4-Dimethylphenyl)thiazol-2-yl]-C-phenyl-methanesulfonamide (23)**

To a 0 °C solution of compound **3** (116 mg, 0.57 mmol) in THF (5 mL) was added Et<sub>3</sub>N (0.23 mL, 1.64 mmol) and DMAP (10 mg, 0.08 mmol). Then, BnSO<sub>2</sub>Cl (130 mg, 0.68 mmol) was added in one portion. The resultant mixture was warmed up to room temperature and stirred for 1.5h. The reaction was quenched with H<sub>2</sub>O (5ml) and H<sub>2</sub>O (30 mL) was added. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of all the solvent, the residue was purified by silica gel chromatography (Hexane / EtOAc = 5 : 1 to 2 : 1) to afford product **23** (129 mg, 63%) as a solid. mp 162-163 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.20 (br, 1H), 7.42-7.40 (m, 2H), 7.30-7.24 (m, 3H), 7.05-7.01 (m, 3H), 6.08 (s, 1H), 4.29 (s, 2H), 2.34 (s, 3H), 2.22 (s, 3H); <sup>13</sup>C NMR (100.5 MHz, CDCl<sub>3</sub>) δ 170.6, 140.1, 136.6, 136.3, 132.0, 131.4, 129.9, 129.1, 128.6, 128.5, 127.3, 126.1, 103.3, 60.2, 21.4, 20.5; MS (ESI) *m/z* 359 (M + H<sup>+</sup>), 381 (M + Na<sup>+</sup>); HRMS Calcd for C<sub>18</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S (M + H<sup>+</sup>), 359.0888 Found: 359.0880.

**N-[4-(2,4-Dimethylphenyl)thiazol-2-yl]-N-methylbenzamide (24)**

To a cooled mixture of NaH (20 mg, 60% in oil, 0.5 mmol) in THF (5 mL) was added a solution of compound **INH1** (120 mg, 0.39 mmol) in THF (3 mL) dropwise. The mixture was warmed up to room temperature and stirred for 20 min. After that, the mixture was cooled to 0 °C again and MeI (32 μL, 0.5 mmol) was added dropwise. Then, the mixture was warmed up to room temperature and stirred for 2h. Then, H<sub>2</sub>O (2 mL) was added to quench the reaction and the mixture was diluted with H<sub>2</sub>O (30 mL). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 15 mL).

The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of all the solvent, the residue was purified by silica gel chromatography (Hexane / EtOAc = 4 : 1) to afford compound **24** (93 mg, 74%) as a solid. mp 146-147 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.40 (m, 2H), 7.46 (m, 3H), 7.15 (m, 3H), 6.49 (s, 1H), 3.56 (s, 3H), 2.41 (s, 3H), 2.16 (s, 3H); <sup>13</sup>C NMR (100.5 MHz, CDCl<sub>3</sub>) δ 174.40, 168.66, 140.44, 138.69, 137.94, 137.30, 131.61, 131.52, 130.77, 129.43, 128.25, 127.47, 127.22, 106.75, 34.14, 21.53, 19.77; MS (ESI) *m/z* 323 (M + H<sup>+</sup>); HRMS Calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>OSNa (M + H<sup>+</sup>), 345.1038 Found: 345.1042.

**2,4-Dimethoxy-*N*-methyl-*N*-[4-(2,4,6-trimethylphenyl)thiazol-2-yl]benzamide (26) and 2,4-dimethoxy-*N*-[3-methyl-4-(2,4,6-trimethylphenyl)-3H-thiazol-2(2Z)-ylidene]benzamide (27)**

To a cooled mixture of NaH (100 mg, 60% in oil, 2.5 mmol) in THF (5 mL) was added a solution of compound **INH2** (140 mg, 0.37 mmol) in THF (5 mL) dropwise. The mixture was warmed up to room temperature and stirred for 30 min. After that, the mixture was cooled to 0 °C again and MeI (1.0 mL, 16.1 mmol) was added dropwise. Then, the mixture was warmed up to room temperature and stirred for 2h. Then, H<sub>2</sub>O (2 mL) was added to quench the reaction and the mixture was diluted with H<sub>2</sub>O (30 mL). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 30 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of all the solvent, the residue was purified by silica gel chromatography (Hexane / EtOAc = 4 : 1 to 2 : 1) to afford compound **26** (less polar, 38 mg, 26%) as a solid and compound **27** (more polar, 93 mg, 63%) as a solid. Compound **26**: mp 156-157°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.35 (d, *J* = 8.4 Hz, 1H), 6.94 (s, 2H), 6.79 (s, 1H), 6.59 (dd, *J* = 10.4, 2.0 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.55 (s, 3H), 2.32 (s, 3H), 2.15 (s, 6H); <sup>13</sup>C NMR (125MHz, CDCl<sub>3</sub>) δ 168.9, 162.9, 157.4, 148.5, 137.8, 137.6, 133.0, 131.1, 130.1, 128.5, 117.6, 112.6, 105.2, 98.8, 55.8, 55.7, 37.1, 21.3, 20.7; MS (ESI) *m/z* 397 (M + H<sup>+</sup>), 419 (M + Na<sup>+</sup>); HRMS Calcd for C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>SNa (M + Na<sup>+</sup>), 419.1405 Found: 419.1390. Compound **27**: mp 161-162 °C; 8.28 (d, *J* = 9.6 Hz, 1H), 6.99 (s, 2H), 6.54 (s, 1H), 6.53 (d, *J* = 6.4 Hz, 1H), 6.39 (s, 1H), 3.95 (s, 3H), 3.87 (s, 3H), 3.44 (s, 3H), 2.35 (s, 3H), 2.07 (s, 6H); <sup>13</sup>C NMR (125MHz, CDCl<sub>3</sub>) δ 173.9, 168.1, 139.9, 138.5, 136.8, 134.0, 128.7, 127.3, 119.8, 106.2, 104.3, 99.1, 56.2, 55.6, 33.4, 21.4, 20.1; MS (ESI) *m/z* 397 (M + H<sup>+</sup>), 419 (M + Na<sup>+</sup>); HRMS Calcd for C<sub>22</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub>S (M + H<sup>+</sup>), 397.1586 Found: 397.1584.

**{Benzoyl-[4-(2,4,6-trimethylphenyl)thiazol-2-yl]-amino}acetic acid ethyl ester (30)**

NaH (38 mg, 60 in oil, 0.95 mmol) was cooled to 0 °C and a solution of compound **6** (167 mg, 0.52 mmol) in DMF (6 mL) was added. The mixture was carefully heated to 90 °C and stirred for 1h. Then, the mixture was cooled to 0 °C and BrCH<sub>2</sub>CO<sub>2</sub>Et (0.60 mL, 5.4 mmol) was added dropwise. After that, the mixture was heated to 90 °C and stirred overnight. H<sub>2</sub>O (10 mL) was added to quench the reaction and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of all the solvent in vacuo resulted in a residue, which was purified with silica gel chromatography (Hexane / EtOAc = 5 : 1) to give compound **30** as a white solid (152 mg, 72%). mp 160-161 °C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.32-8.30 (m, 2H), 7.49 (tt, *J* = 7.0, 1.5 Hz, 1H), 7.45-7.42 (m, 2H), 6.99 (s, 2H), 6.47 (s, 1H), 4.50 (s, 2H), 4.16 (q, *J* = 7.0 Hz, 2H), 2.36 (s, 3H), 2.13 (s, 6H), 1.20 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C NMR (125.5 MHz, CDCl<sub>3</sub>) δ 174.1, 168.6, 167.3, 140.4, 139.0, 136.9, 136.5, 131.8, 129.5, 129.0, 128.2, 126.0, 106.5, 61.9, 47.9, 21.4, 20.1, 14.3; MS (ESI) *m/z* 409 (M + H<sup>+</sup>), 431 (M + Na<sup>+</sup>); HRMS Calcd for C<sub>23</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub>S (M + H<sup>+</sup>), 409.1586 Found: 409.1594.

**{Benzoyl-[4-(2,4,6-trimethylphenyl)thiazol-2-yl]amino}acetic acid (31)**

Compound **30** (75 mg, 0.18 mmol) was dissolved in CH<sub>3</sub>CN (6 mL). Then, MeOH (3 mL), H<sub>2</sub>O (1.5 mL) and LiOH·H<sub>2</sub>O (30 mg, 0.71 mmol) were added subsequently. The resultant mixture was stirred at room temperature for 4h. H<sub>2</sub>O (20 mL) was added to quench the reaction

and HCl (1M, 3 mL) was added. The resultant mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of all the solvent in vacuo resulted in a residue, which was purified with silica gel chromatography (Hexane / EtOAc = 2 : 1 to 1 : 2) to give compound **31** (53 mg, 77%) as a white solid. mp 198-199 °C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 10.40 (br, 1H), 8.30 (d, *J* = 7.5 Hz, 2H), 7.49-7.39 (m, 3H), 7.00 (s, 2H), 6.48 (s, 1H), 4.59 (2H), 2.38 (s, 3H), 2.10 (s, 6H); <sup>13</sup>C NMR (100.5 MHz, CDCl<sub>3</sub>) δ 174.1, 171.1, 168.8, 140.4, 138.8, 136.6, 136.2, 131.8, 129.4, 128.9, 128.2, 125.5, 106.9, 47.7, 21.3, 19.9; MS (ESI) *m/z* 381 (M + H<sup>+</sup>), 403 (M + Na<sup>+</sup>); 761 (2M + H<sup>+</sup>), 783 (M + 2Na<sup>+</sup>); HRMS Calcd for C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>Na (M + Na<sup>+</sup>), 403.1092 Found: 403.1093.

### 2-Hydroxy-4-methoxy-*N*-[4-(2,4,6-trimethylphenyl)thiazol-2-yl]benzamide (**32**) and 2,4-dihydroxy-*N*-[4-(2,4,6-trimethylphenyl)thiazol-2-yl]benzamide (**33**)

To a 0°C solution of **INH2** (148 mg, 0.39 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL) was added BBr<sub>3</sub> (2.5 mL, 1M in CH<sub>2</sub>Cl<sub>2</sub>, 2.5 mmol) dropwise. The mixture was warmed up to room temperature and stirred for 3h. Then, MeOH (1 mL) was added and all the solvent was removed in vacuo. The procedure of addition of MeOH (1 mL) was repeated four times. The residue was purified by silica gel chromatography (hexane / EtOAc = 4 : 1) to give compound **32** (less polar, 28 mg, 20%) as a solid and compound **33** (more polar, 88mg, 64%) as a solid. Compound **32**: mp 206-207 °C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 11.83 (s, 2H), 7.47 (s, 1H), 6.75 (s, 1H), 6.68 (s, 2H), 6.33 (dd, *J* = 2.0, 2.0 Hz, 1H), 6.22 (s, 1H), 3.78 (s, 3H), 2.22 (s, 3H), 1.98 (s, 6H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 167.15, 165.02, 162.51, 159.27, 147.98, 138.19, 137.04, 130.88, 129.57, 128.34, 111.62, 107.47, 106.91, 101.54, 55.61, 21.16, 20.51; MS (ESI) *m/z* 369 (M + H<sup>+</sup>); HRMS Calcd for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>Na (M + Na<sup>+</sup>), 391.1092 Found: 325.1082. Compound **33**: mp 236.5-237.5 °C; <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>) δ 11.83 (s, 2H), 10.31 (s, 1H), 7.93 (d, *J* = 6.4 Hz, 1H), 6.98 (s, 1H), 6.91 (s, 2H), 6.42 (d, *J* = 6.4 Hz, 2H), 2.25 (s, 3H), 2.05 (s, 6H); <sup>13</sup>C NMR (100.5 MHz, DMSO-D<sub>6</sub>) δ 164.33, 163.08, 159.88, 156.88, 148.24, 136.62, 132.48, 131.85, 127.89, 111.16, 108.28, 107.38, 102.74, 20.69, 20.04; MS (ESI) *m/z* 355 (M + H<sup>+</sup>); HRMS Calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>Na (M + Na<sup>+</sup>), 377.0936 Found: 377.0932.

### 4-(2-Methoxy-4,6-dimethylphenyl)thiazol-2-ylamine (**35**)

To a solution of compound **34** (500 mg, 3.05 mmol) in acetone (30 mL) was added K<sub>2</sub>CO<sub>3</sub> (7.6 g, 55 mmol) and Me<sub>2</sub>SO<sub>4</sub> (4.5 mL, 47.5 mmol). The resultant mixture was heated to reflux for 4h. Then, the mixture was cooled to room temperature and NaOH (2N, 100 mL) was added. The whole mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 60 mL) and the combined organic phases were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of all the solvent in vacuo gave a residue, which was dissolved in dioxane (8 mL). To the resultant solution, HBr (0.6 mL, 5.3 mmol) and <sup>t</sup>BuOOH (0.6 mL, 5.0-6.0M in hexane, ~3.4 mmol) were added subsequently. The mixture was refluxed overnight. After cooled to room temperature, all the solvent was removed in vacuo to give a residue. The residue was dissolved in H<sub>2</sub>O (20 mL) and the whole mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 60 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. All the solvent was removed in vacuo and the resultant residue was purified by silica gel chromatography (hexane / EtOAc = 100 : 1) to give a compound (169 mg) as a oil. This oil (169 mg) was dissolved in EtOH (4 mL) and thiourea (59 mg, 0.78 mmol) was added. The resultant mixture was heated to reflux for 1h. After cooled to room temperature, all the solvent was removed in vacuo to provide a residue. To this residue, saturated aqueous NaHCO<sub>3</sub> (15 mL) was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 15 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. All the solvent was removed in vacuo and the resultant residue was purified by silica gel chromatography (hexane / EtOAc = 2 : 1 to 1 : 1) to give compound **35** (140 mg, 20%) as a solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.68 (t, *J* = 0.8 Hz, 1H), 6.60 (s, 1H), 6.35 (s, 1H), 5.05 (s, 2H), 3.74 (s, 3H), 2.34 (s, 3H), 2.17 (s, 3H); <sup>13</sup>C NMR (100.5 MHz, CDCl<sub>3</sub>) δ 166.65, 158.02, 147.15, 139.11, 138.93, 123.36, 121.95, 109.47,

107.12, 56.07, 21.84, 20.32; MS (ESI)  $m/z$  235.17 ( $M + H^+$ ); HRMS Calcd for  $C_{12}H_{15}N_2OS$  ( $M + H^+$ ), 235.0905 Found: 235.0907.

### ***N*-[4-(2,4-Dimethylphenyl)oxazol-2-yl]benzamide (39)**

The mixture of compound **1** (1.0 g, 4.41 mmol) and urea (290 mg, 4.79 mmol) in anhydrous EtOH (17 mL) was heated to reflux for 4.0 h. After that, the solvent was removed in vacuo and the aqueous saturated  $NaHCO_3$  was added to make the mixture base (PH =8-9). Then, the mixture was extracted with  $CH_2Cl_2$  ( $3 \times 30$  mL). The combined organic phases were dried with anhydrous  $Na_2SO_4$ . After removal of all the solvent, the residue was purified by silica gel chromatography (Hexane / EtOAc = 3 : 1) to afford the desired amine (100 mg) as a solid. A cooled solution of the above solid (100 mg) and DMAP (129 mg, 1.06 mmol) in  $CH_2Cl_2$  (3 mL) was added a solution of BzCl (100 mg, 0.71 mmol) in  $CH_2Cl_2$  (1 mL) dropwise. The mixture was warmed up to room temperature and stirred for 40min. After that, the mixture was poured into  $H_2O$  (20 mL) and the resultant mixture was extracted with  $CH_2Cl_2$  ( $3 \times 20$  mL). The combined organic phases were dried with anhydrous  $Na_2SO_4$ . After removal of all the solvent, the residue was purified by silica gel chromatography (Hexane / EtOAc = 8 : 1) to give the compound **39** (92 mg) as a solid. mp 137-138 °C;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  10.77 (br, 1H), 7.88 (s, 2H), 7.55 (s, 1H), 7.46 (s, 2H), 7.28 (s, 2H), 7.06-7.10 (m, 2H), 2.39 (s, 3H), 2.33 (s, 3H);  $^{13}C$ -NMR (125 MHz,  $CDCl_3$ )  $\delta$  138.6, 135.7, 132.8, 131.9, 128.8, 128.5, 128.2, 127.1, 21.5, 21.3; MS (ESI)  $m/z$  292 ( $M + H^+$ ); HRMS Calcd for  $C_{18}H_{17}N_2O_2$  ( $M + H^+$ ), 293.1290 Found: 293.1298.

### ***N*-[4-(2,4-Dimethylphenyl)thiazol-2-yl]thiobenzamide (40)**

The mixture of **INH1** (152 mg, 0.49 mmol) and Lawesson reagent (210 mg, 0.49 mmol) in toluene (10 mL) was heated to reflux for 2h. After that, the mixture was cooled to room temperature and all the solvent was removed in vacuo. The resultant residue was purified by silica gel chromatography (Hexane / EtOAc = 20 : 1) to give compound **40** (126 mg, 79%) as a solid. mp 161.5-162.5 °C;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  12.37 (br, 1H), 7.68 (d,  $J = 7.6$  Hz, 2H), 7.44 (t,  $J = 7.2$  Hz, 1H), 7.30 (t,  $J = 7.6$  Hz, 2H), 7.20 (d,  $J = 8.0$  Hz, 1H), 6.97-6.93 (m, 2H), 6.88 (s, 1H), 2.34 (s, 3H), 2.29 (s, 3H);  $^{13}C$  NMR (100.5 MHz,  $CDCl_3$ )  $\delta$  195.0, 160.5, 149.6, 141.0, 138.1, 135.7, 131.8, 131.8, 130.9, 129.6, 128.6, 127.3, 126.8, 122.2, 109.7, 21.3, 21.0; MS (ESI)  $m/z$  325 ( $M + H^+$ ), 347 ( $M + Na^+$ ); HRMS Calcd for  $C_{18}H_{16}N_2S_2Na$  ( $M + Na^+$ ), 347.0653 Found: 347.0644.

## **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

## **Acknowledgements**

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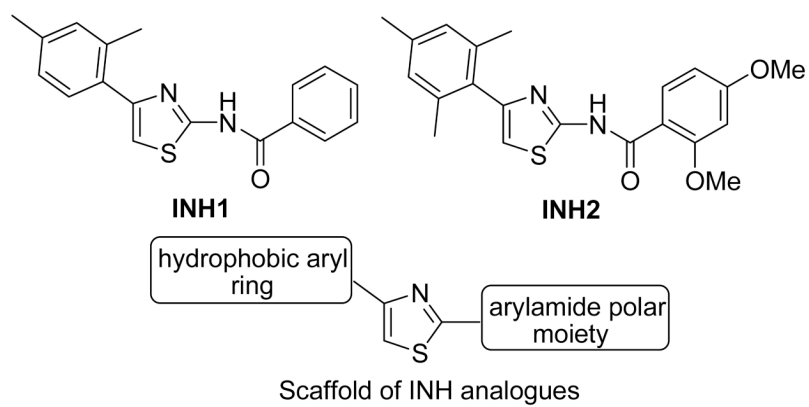
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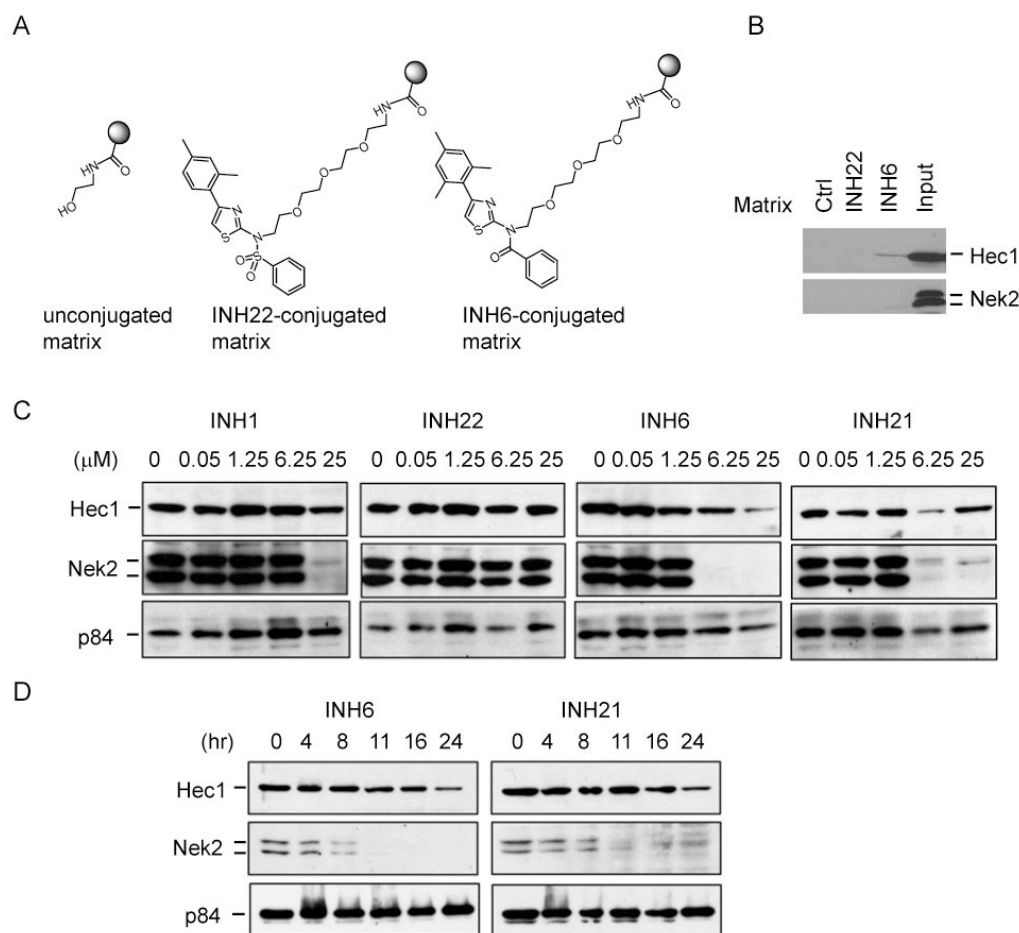


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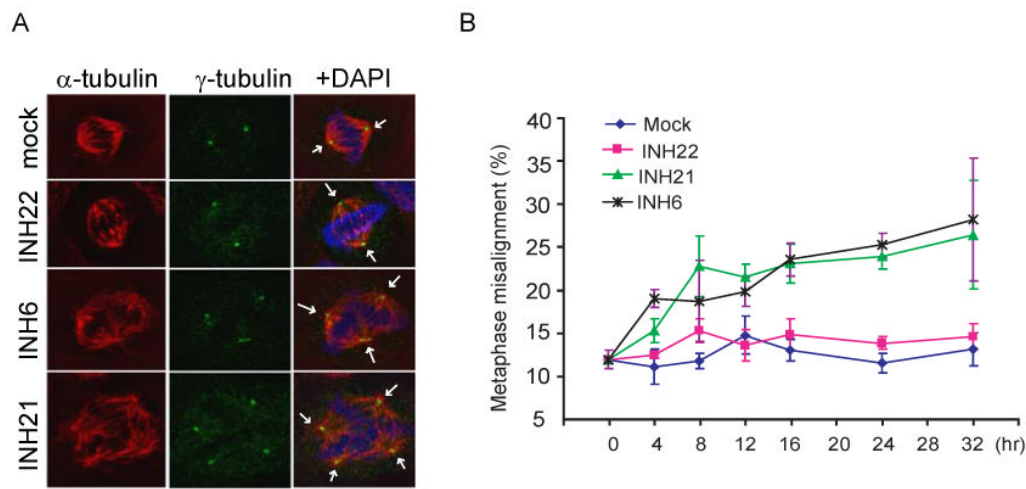




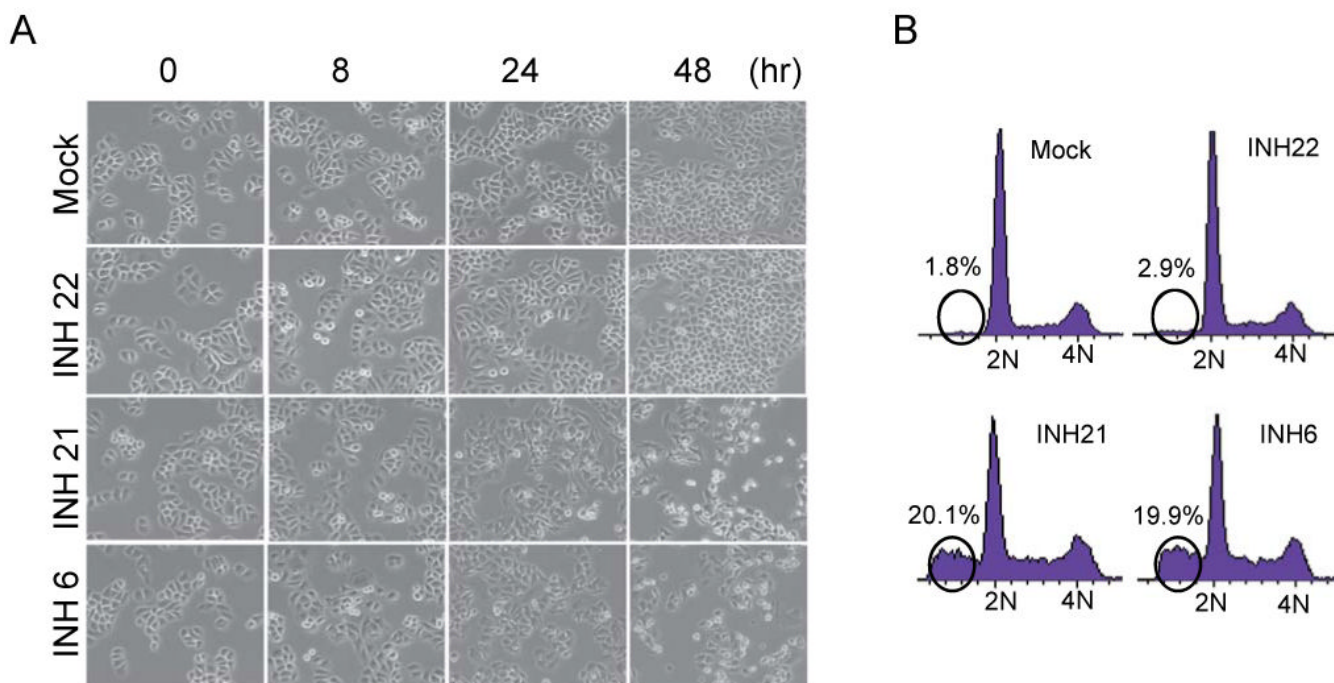
**Figure 1.**  
Structures of **INH1** and **INH2** and the scaffold of INH analogues

**Figure 2.**

Target validation. (A) Illustrations of compound conjugated affi-gel matrices. (B) HeLa cell extract was used for affinity binding by control matrix, INH22, or INH6 conjugated matrix. Resultant precipitates after sufficient washing were subjected to SDS-PAGE analysis and Western blot to detect Hec1 and Nek2. Whole cell extract was used as input in duplicates. (C) dosage-dependent kinetic study of compound treatment. HeLa cells were treated with 6.25  $\mu$ M INH6 or INH21, and harvested at various time points for detecting Hec1 and Nek2 by Western blot. P84 is a nuclear matrix protein used a loading control. (D) time-dependent kinetic study with varying dosages of the indicated compounds. Western blot was similarly performed as in (C).

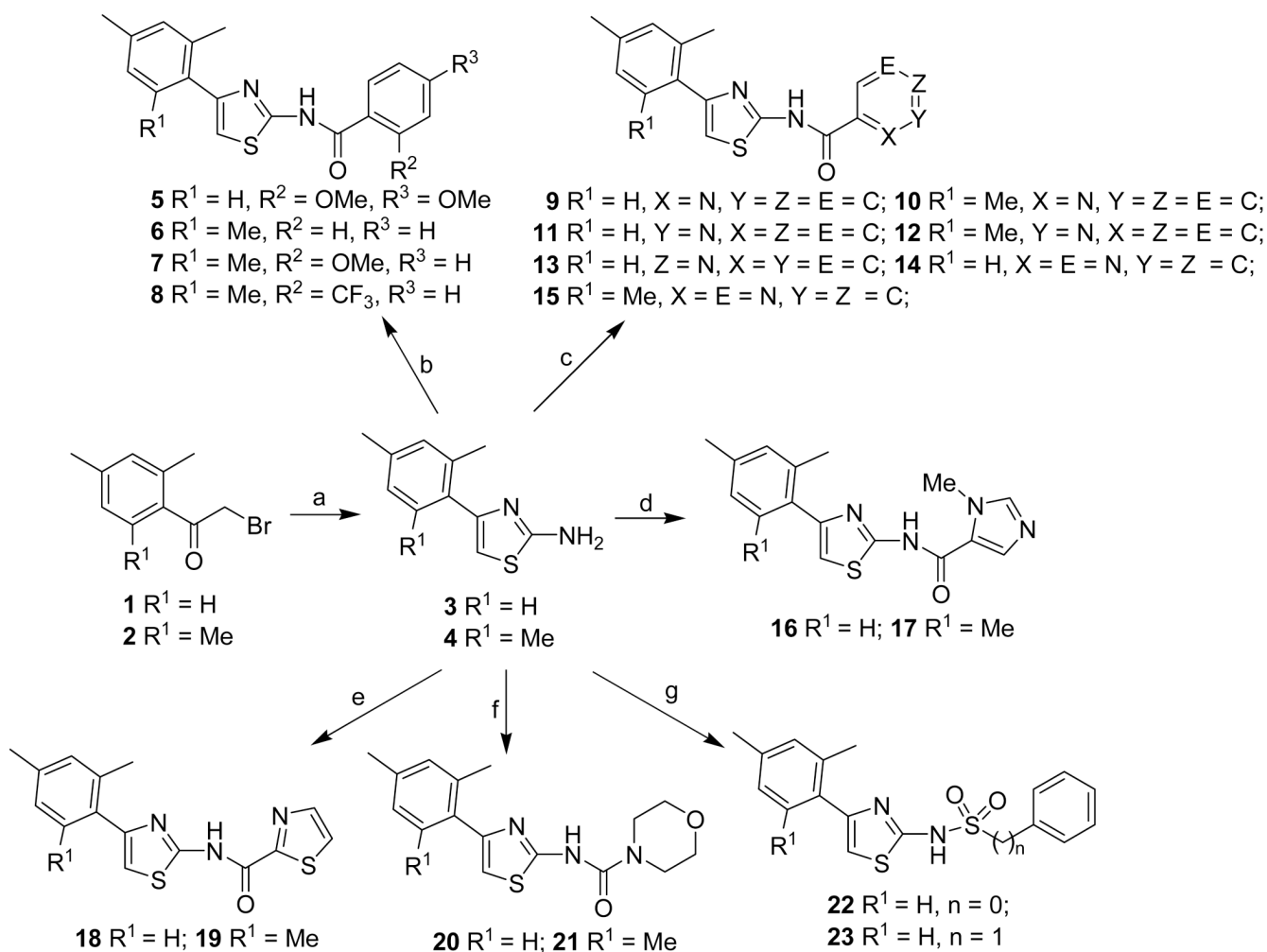


**Figure 3.** Mitotic abnormality induced by compound **6** and **21**, but not **22** and the mock (buffer control). (A) Immunofluorescent staining of HeLa cells treated with mock, compound **22**, **6** or **21** (each at 3.75  $\mu$ M) for 24 hrs. Left column showed merged images of  $\alpha$ -tubulin,  $\gamma$ -tubulin and the DAPI dye (DNA). Arrows indicated spindle poles. Note the multipolar spindle configurations in **6** and **21** treated cells. Scale bar, 10  $\mu$ M. (B) incidence of metaphase chromosome alignment in cells treated individual compounds (2.5  $\mu$ M each). Triplicate sets of compound-treated HeLa cells expressing the chromosome marker H2B-GFP were measured to obtain the averages and deviations.

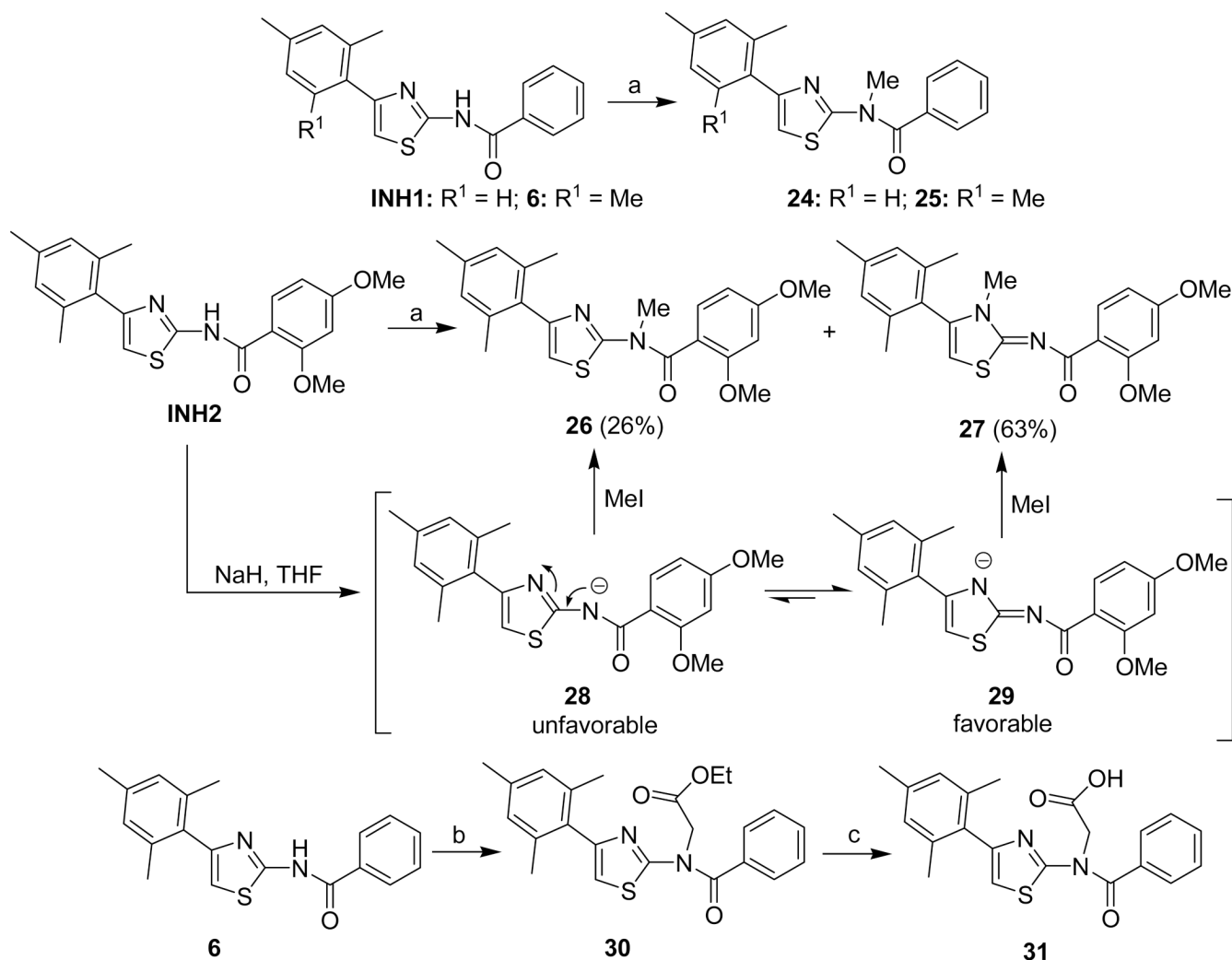


**Figure 4.**

Apoptosis of cells treated with compound **6** and **21**, but not **22** and the mock (buffer control). (A) Phase contrast images of cells at various time points after treatment with mock or individual compounds (each at  $2.5 \mu\text{M}$ ). (B) Percentages of metaphase population with chromosome alignment in cells treated with individual compounds ( $2.5 \mu\text{M}$  each). Triplicate sets of compound-treated HeLa cells expressing the chromosome marker H2B-GFP were measured to obtain the averages and deviations.

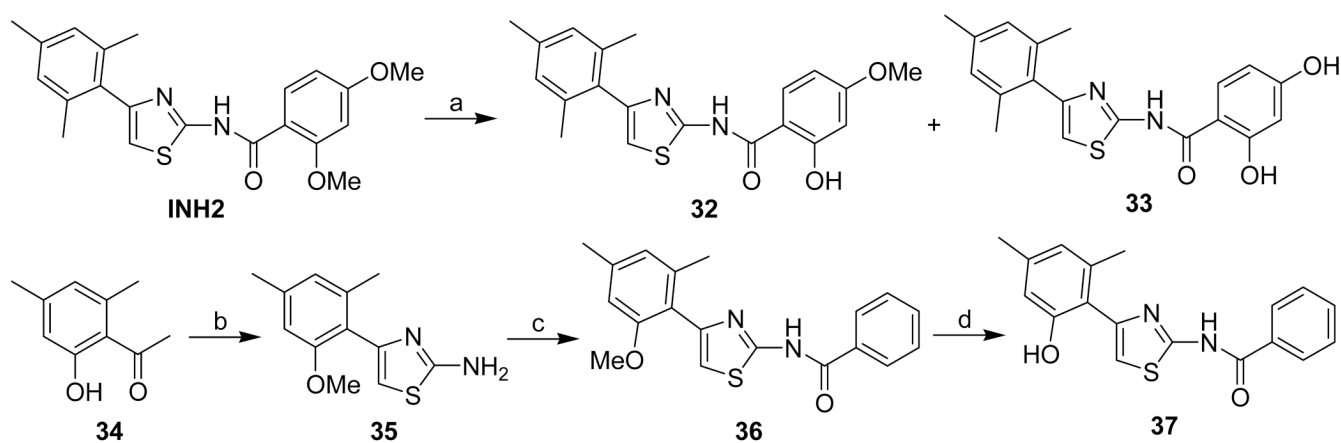
**Scheme 1.**General Synthetic Procedure for the Preparation of Compounds **5-23**<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) Thiourea, EtOH, reflux (89% for **3**, 99% for **4**); (b) for **5**: 2,4-dimethoxy benzoyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt. (67%); for **6**: BzCl, Et<sub>3</sub>N, dioxane, reflux (48%); for **7**: 2-methoxyl benzoyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (77%); for **8**: 2-trifluoromethylbenzoyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (52%); (c) for **9**: picolinoyl chloride hydrochloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (87%); for **10**: picolinoyl chloride hydrochloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (93%); for **11**: nicotinoyl chloride hydrochloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (64%); for **12**: nicotinoyl chloride hydrochloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (73%); for **13**: isonicotinoyl chloride hydrochloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (88%); for **14**: pyrazine-2-carbonyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (77%); for **15**: pyrazine-2-carbonyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (93%); (d) 1-methyl-1H-imidazole-5-carbonyl chloride hydrochloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (37% for **16**, 28% for **17**); (e) 1,3-thiazole-2-carbonyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (76% for **18**, 89% for **19**); (f) 4-morpholinecarbonyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (63% for **20**, 39% for **21**). (g) for **22**: PhSO<sub>2</sub>Cl, DMAP, pyridine, 0°C to rt. (68%); for **23**: BnSO<sub>2</sub>Cl, Et<sub>3</sub>N, DMAP, THF, 0°C to rt. (63%).

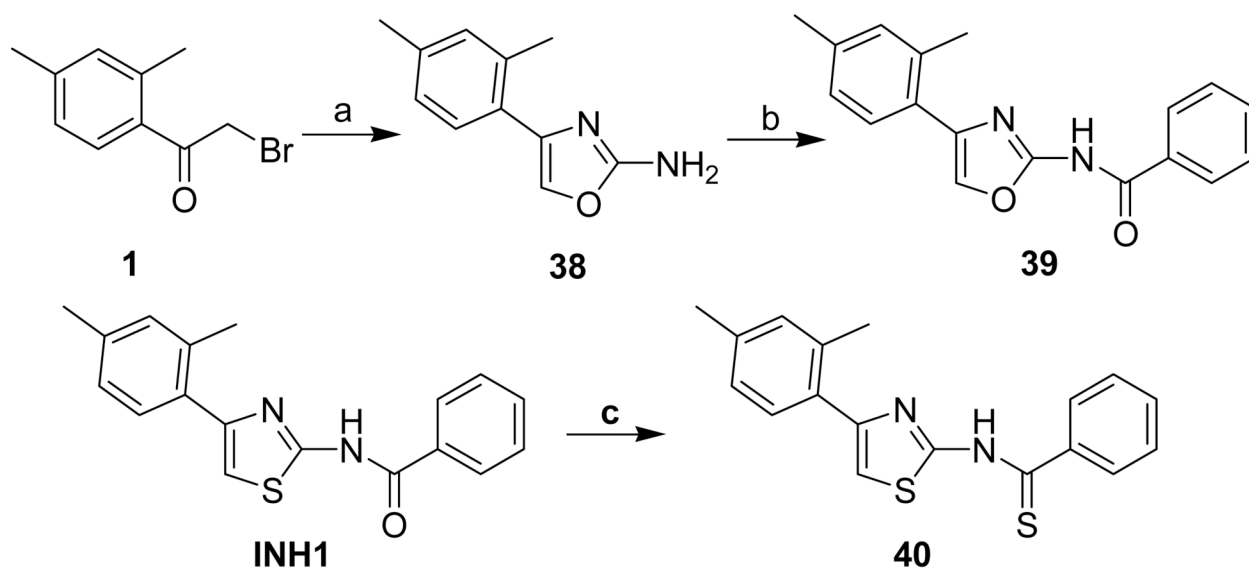
**Scheme 2a.**

<sup>a</sup> Reagents and conditions: (a) (i) NaH, THF, 0°C; (ii) MeI, 0°C to rt. (74% for **24**, 89% for **25**, 26% for **26**, 63% for **27**); (b) (i) NaH, DMF, 0°C to 90°C; (ii) BrCH<sub>2</sub>CO<sub>2</sub>Et, 90°C (72%); (c) LiOH, H<sub>2</sub>O, MeOH, rt. (77%).



**Scheme 3a.**

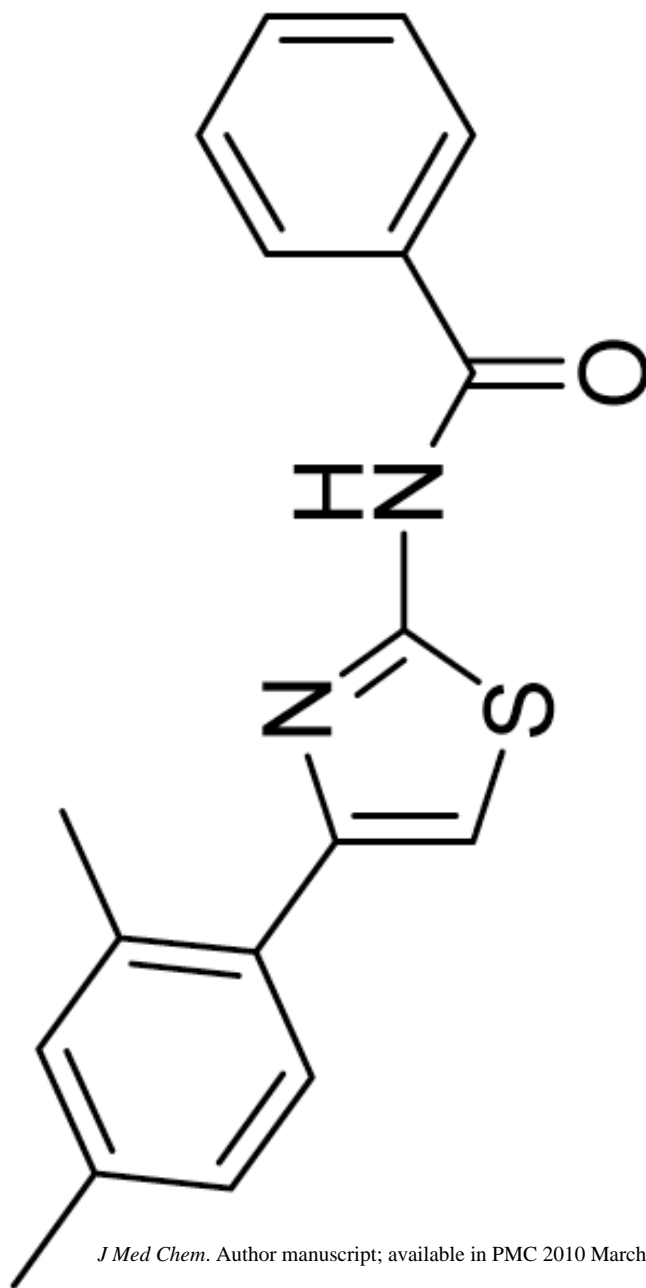
<sup>a</sup> Reagents and conditions: (a) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (20% for **32**, 64% for **33**); (b) (i) Me<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux; (ii) HBr, <sup>t</sup>BuOOH, dioxane, reflux; (iii) thiourea, EtOH, reflux (20% over 3 steps); (c) BzCl, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (93%); (d) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (90%).

**Scheme 4a.**

<sup>a</sup> Reagents and conditions: (a) urea, EtOH, reflux; (b) BzCl, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt. (7% in 2 steps); (c) Lawesson reagent, toluene, reflux (79%).

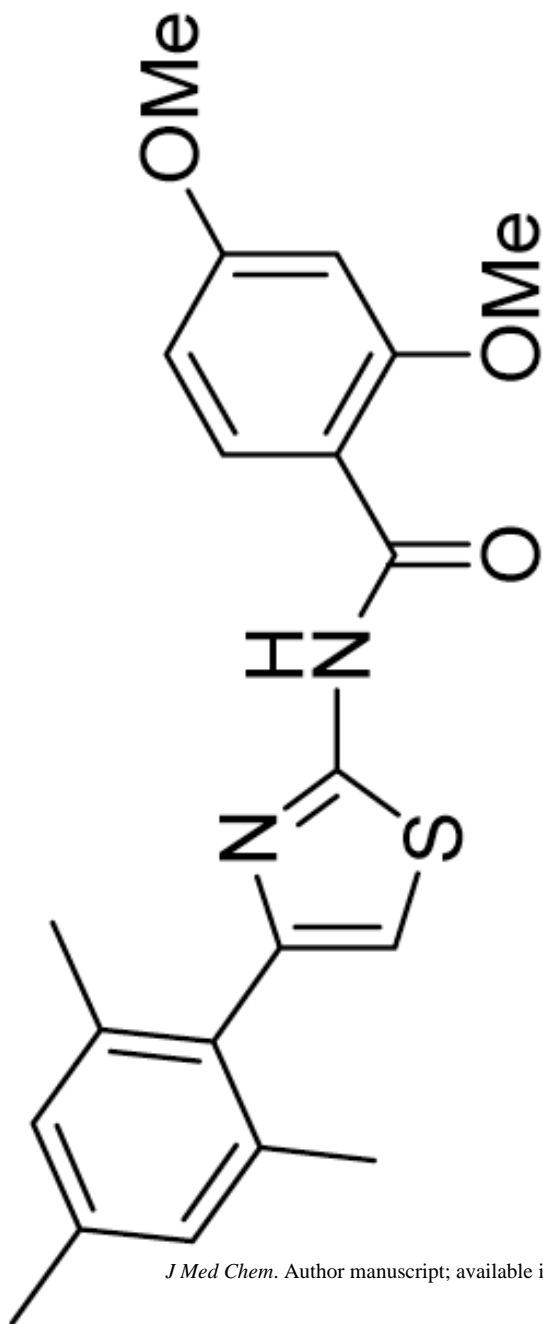
### Activity of INH1, INH2 and their analogues

Compound	IC50 (μM)			
	MB231	MB468	HeL a	K562
Structure				



*J Med Chem.* Author manuscript; available in PMC 2010 March 26.

Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562



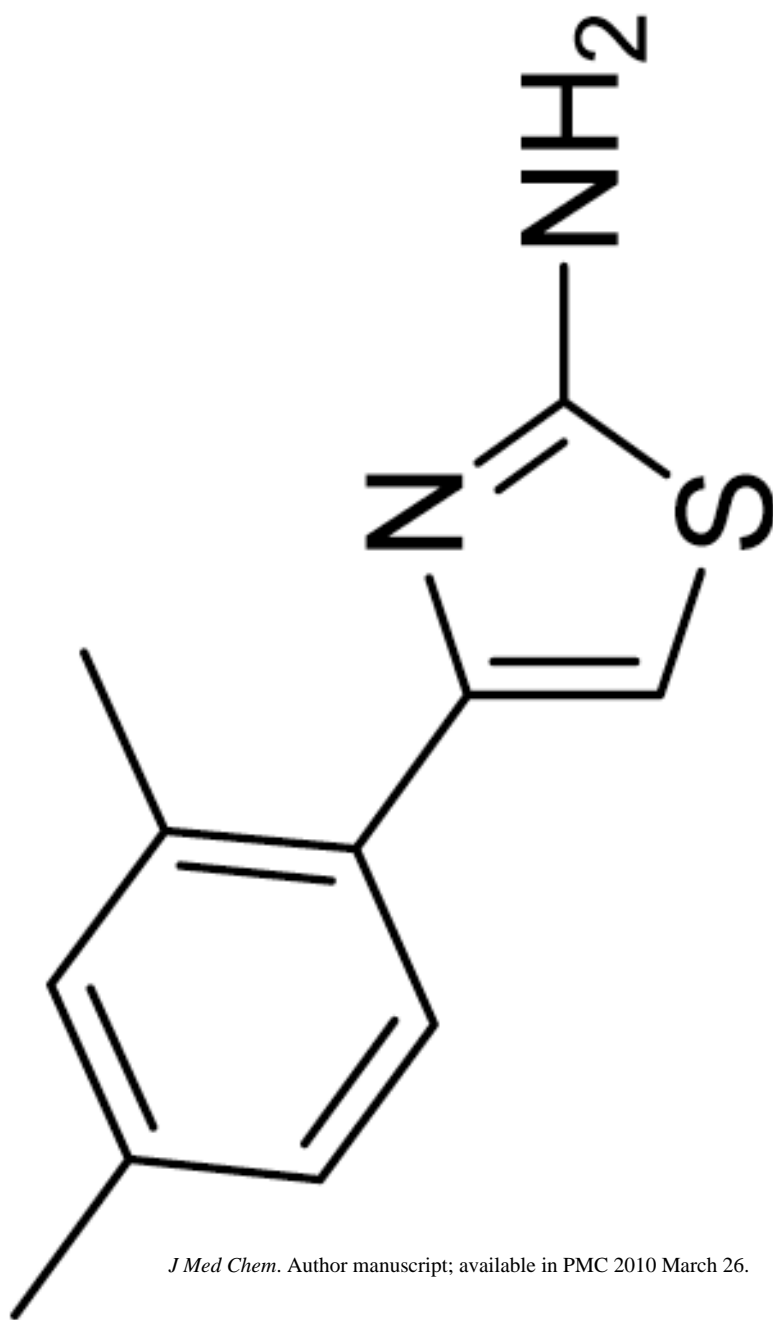
9.7 11.2 13.6 9.5

Compound

Structure

IC50 (μM)

MB231 MB468 HeLa K562



>40 >40 >40 >40

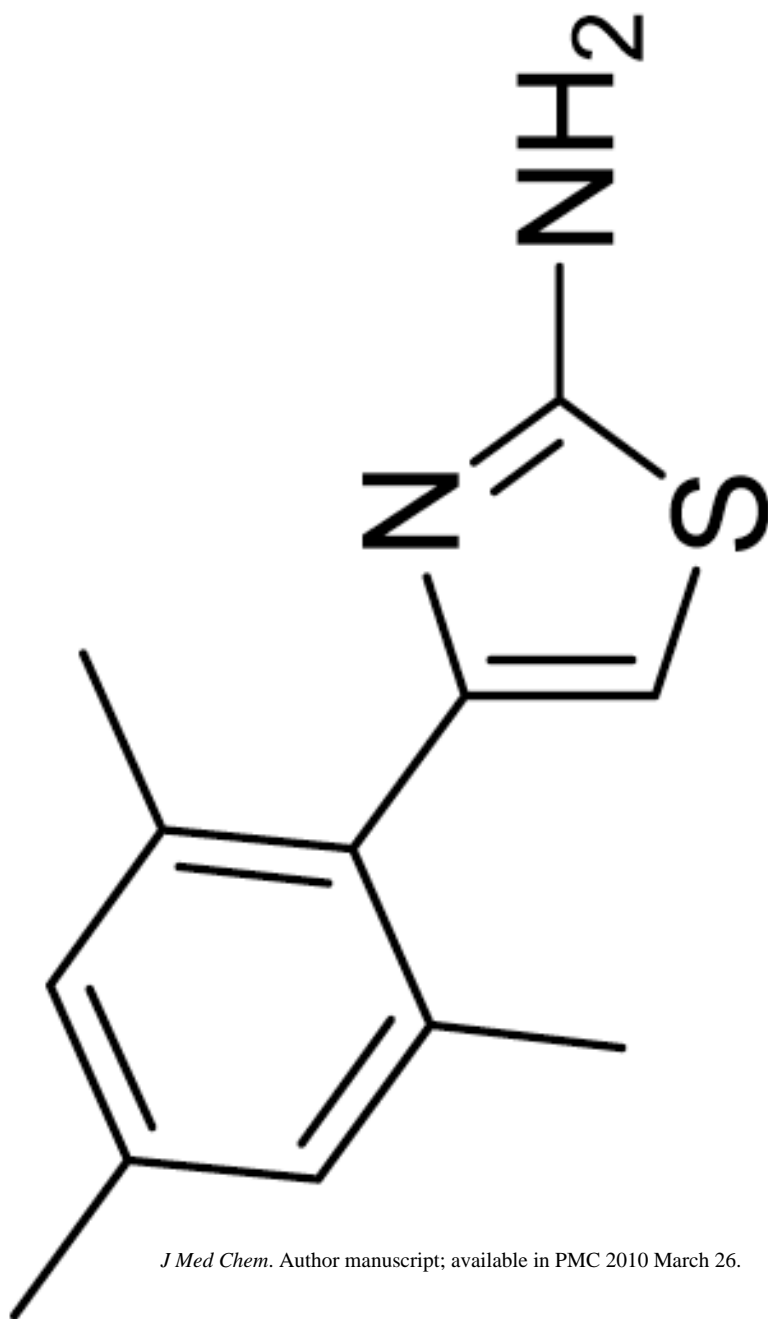
3

Compound

Structure

IC50 (μM)

MB231 MB468 HeLa K562



4

>40

>40

>40

>40

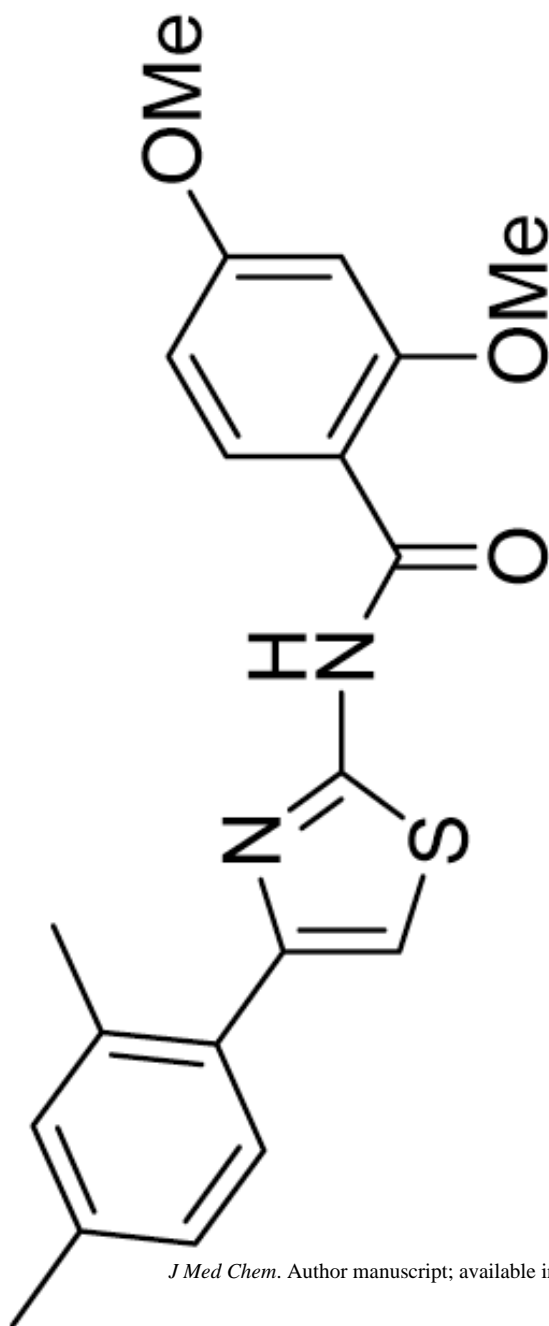


Compound

Structure

IC50 (μM)

MB231 MB468 HeLa K562

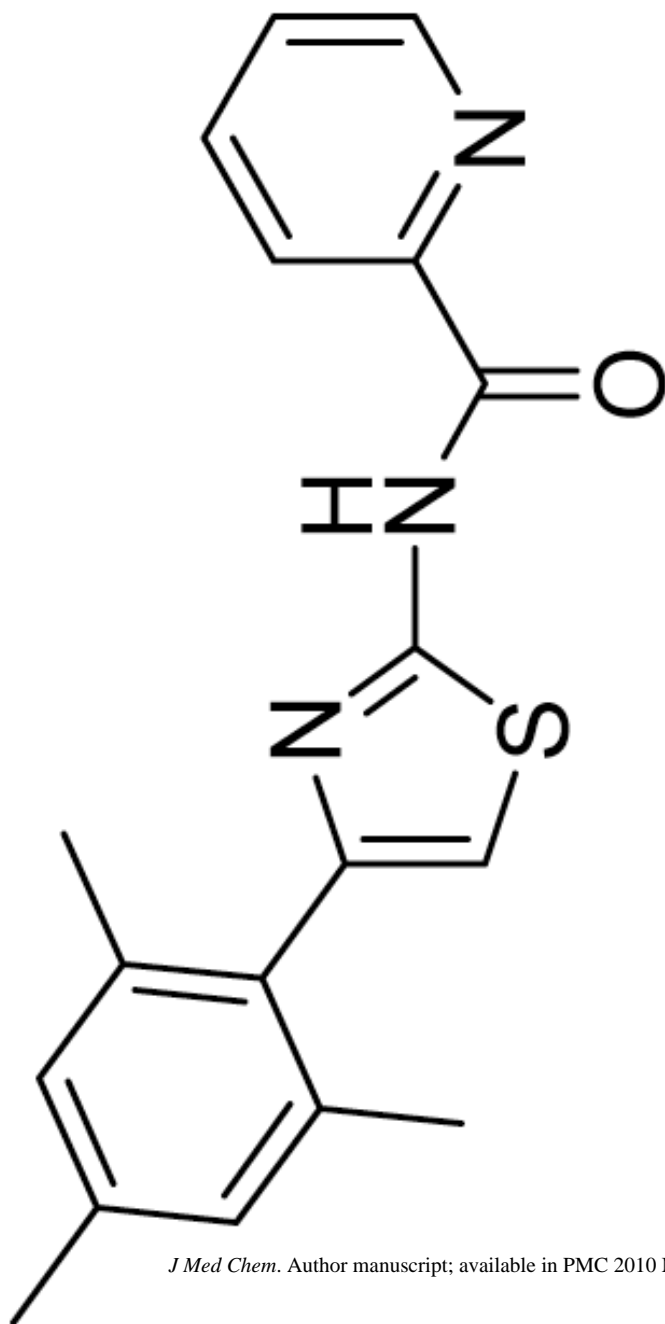


23.4 >40 >40 >40

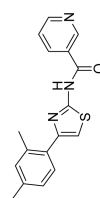
Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562
6		1.7	2.1	2.4	2.5
7		>40	>40	>40	>40
8		5.9	4.1	10.6	7.5
9		7.5	6.2	14.5	13.3

Compound	IC50 (μM)			
	MB231	MB468	HeLa	K562

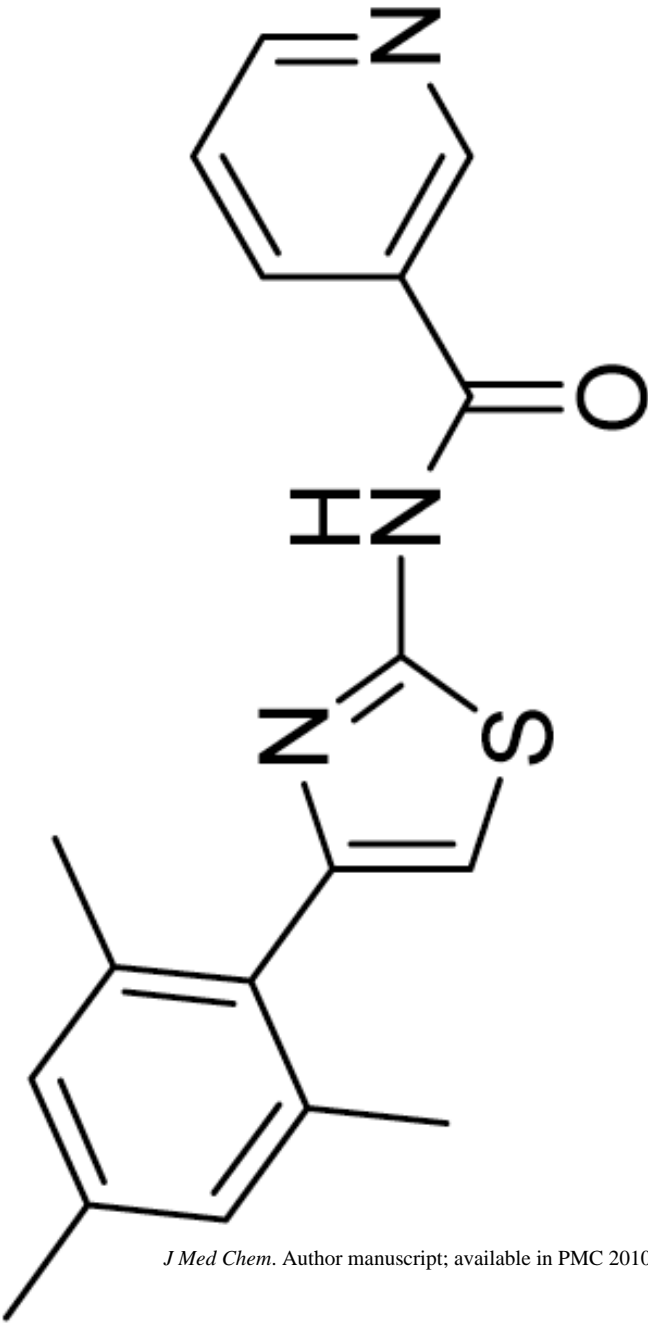
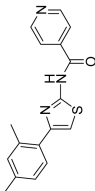
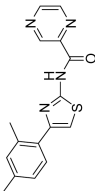
Structure



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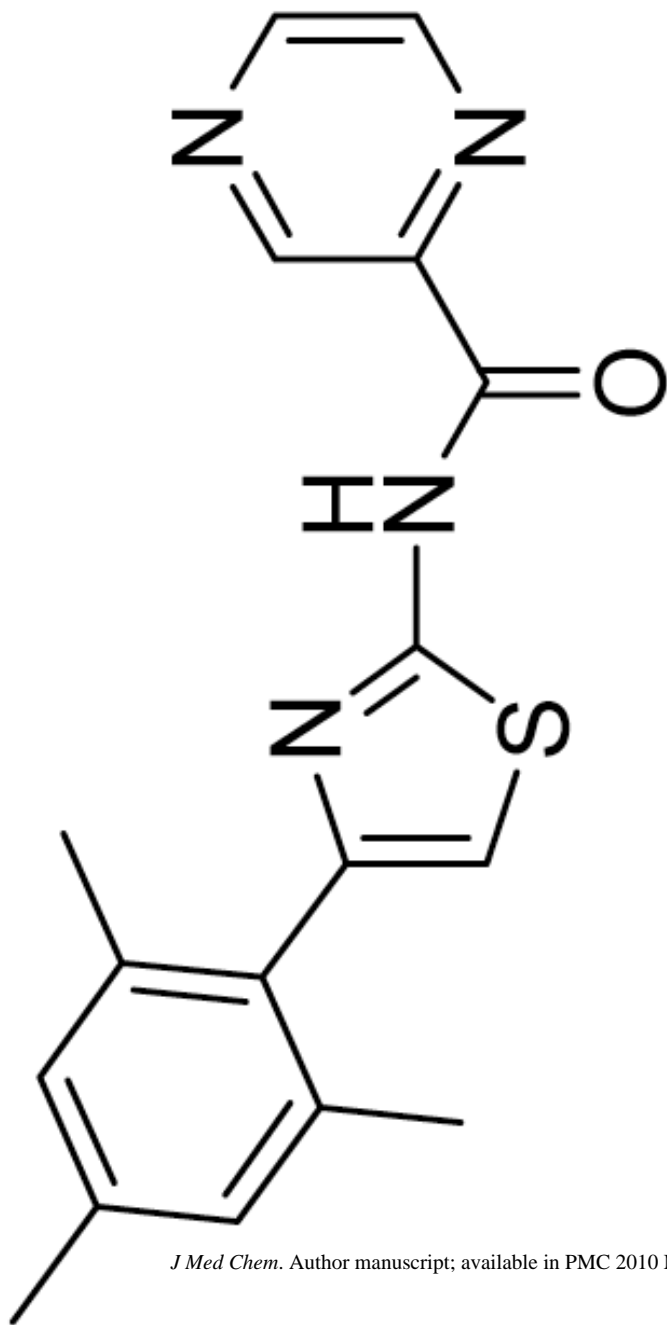


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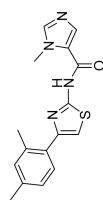
Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562
12		3.2	3.0	3.6	3.9
13		1.1	3.4	1.6	1.2
14		>25	>25	>25	>25

Compound	IC50 (μM)			
	MB231	MB468	HeLa	K562

Structure



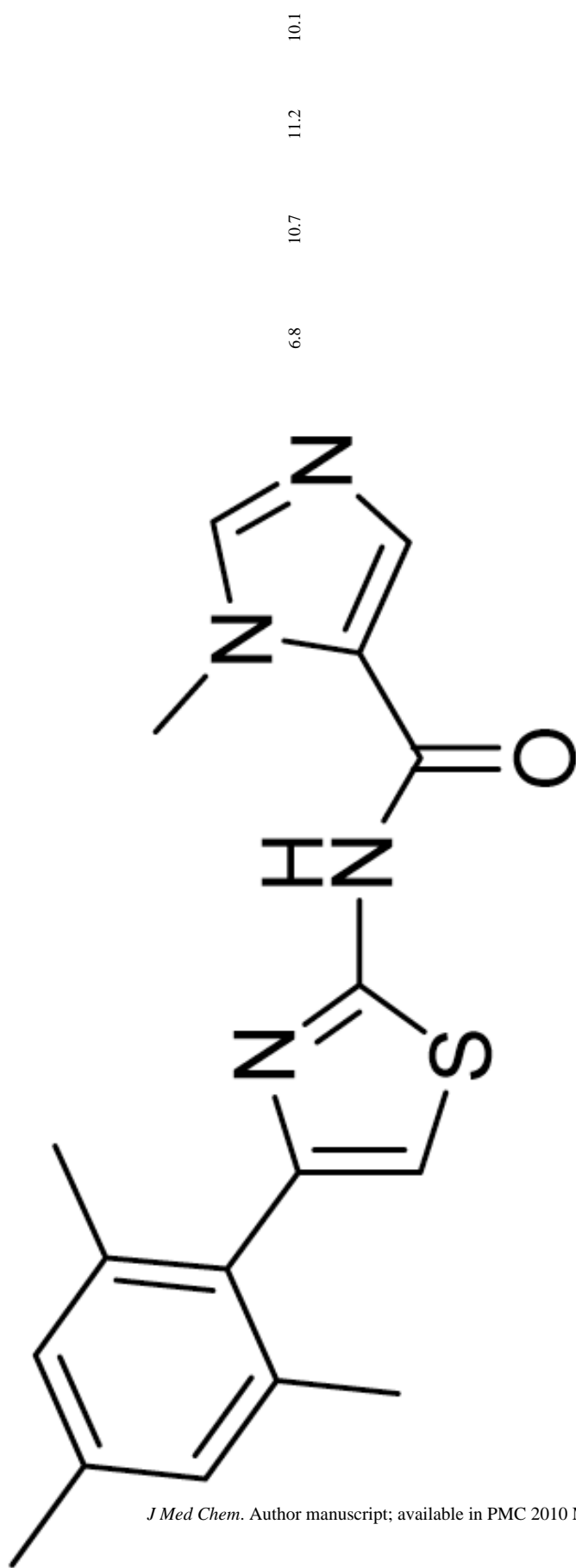
15



16

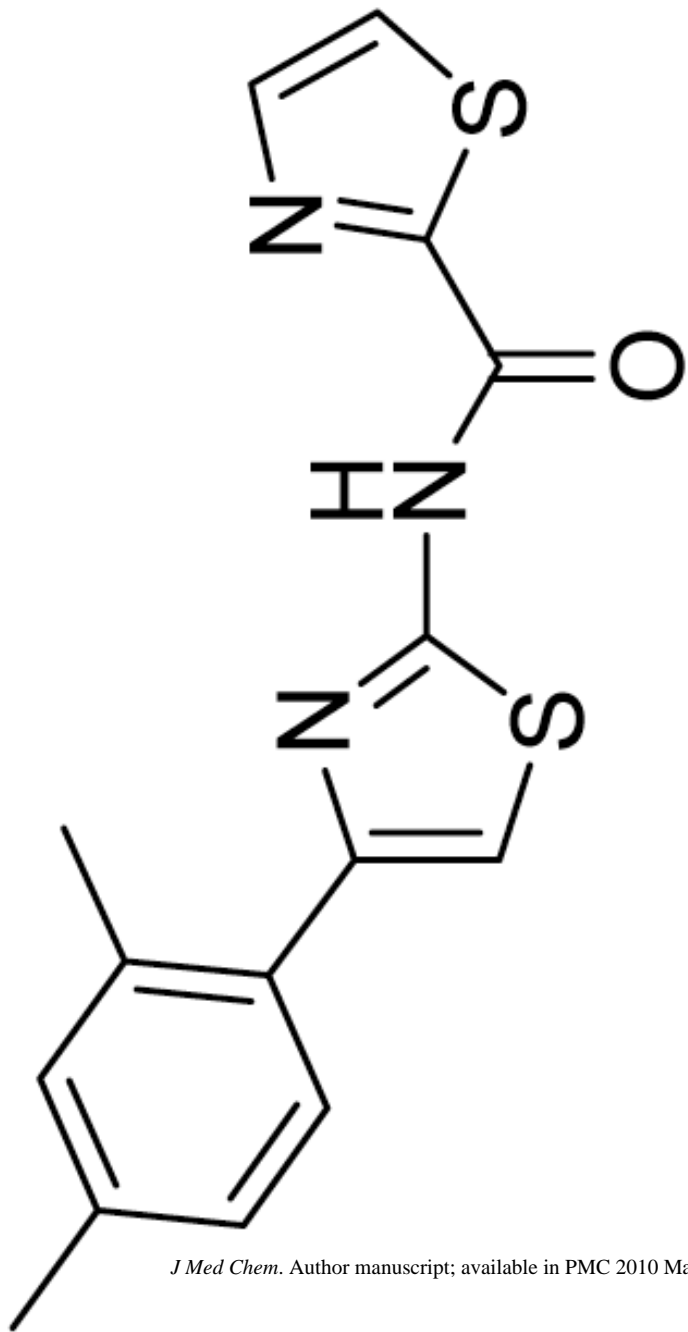
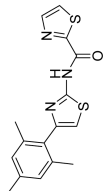
>25 >25 >25 >25

Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562



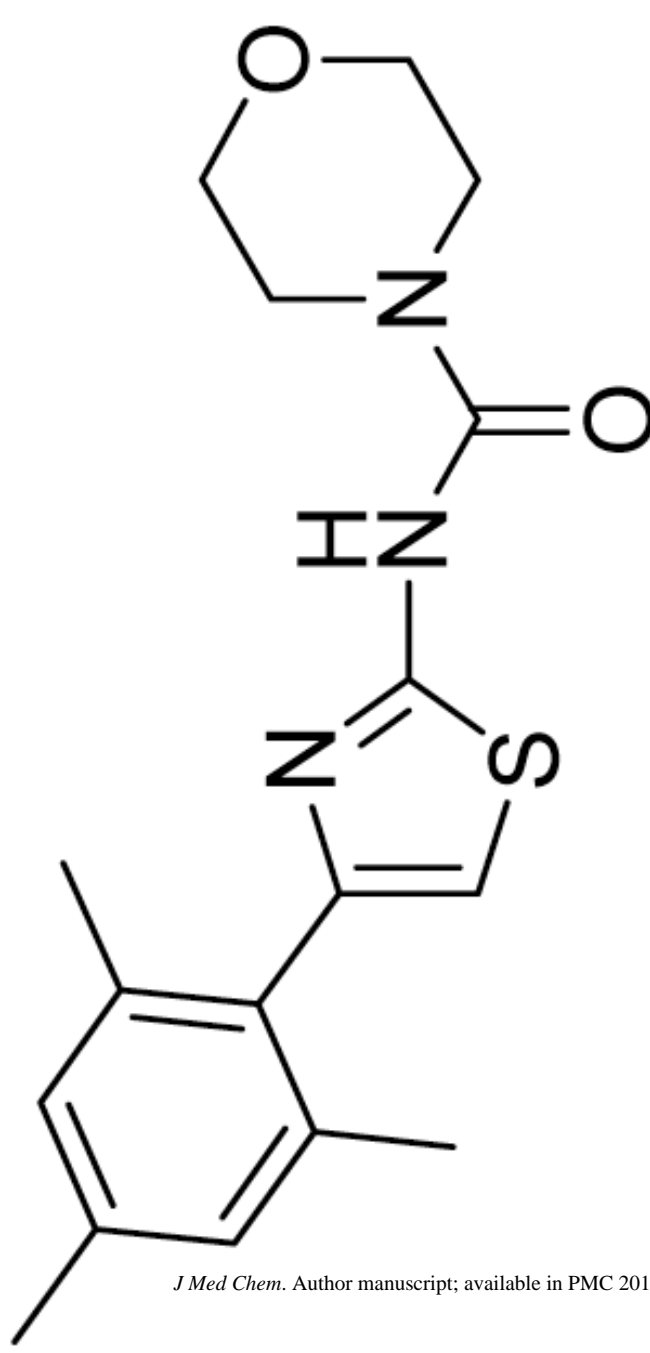
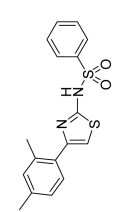


Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562

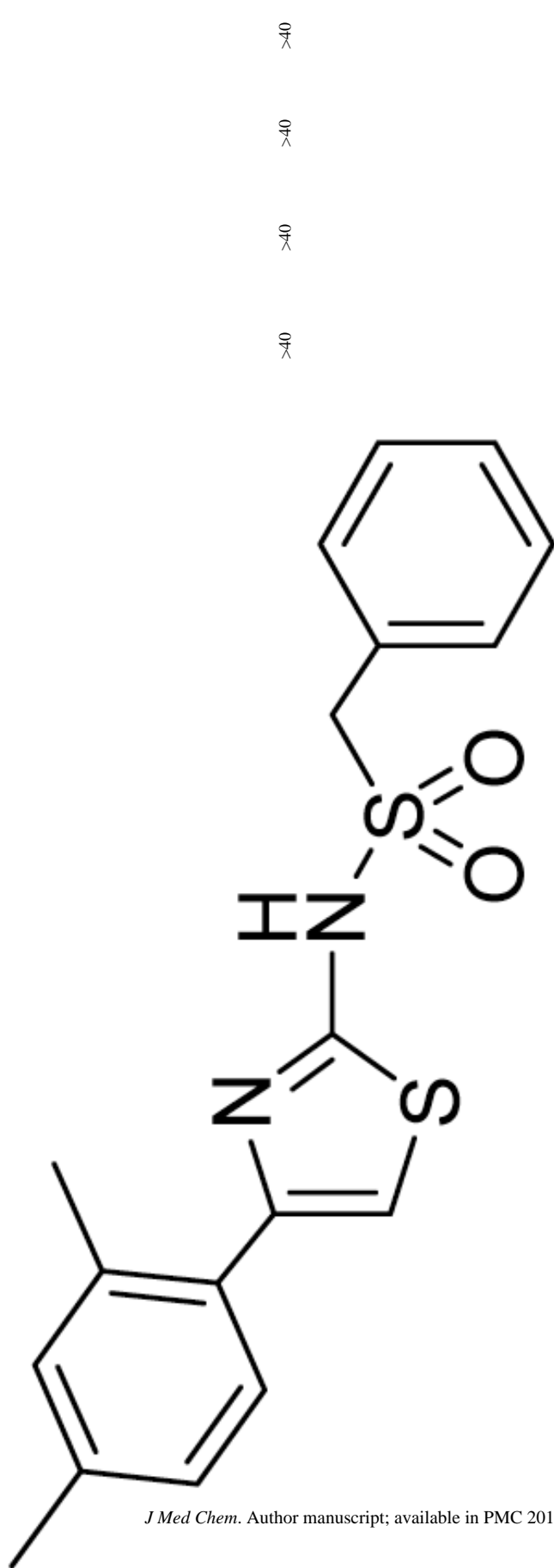
18		2.9	6.2	7.4	10.1
19		4.7	11.8	11.2	14.5



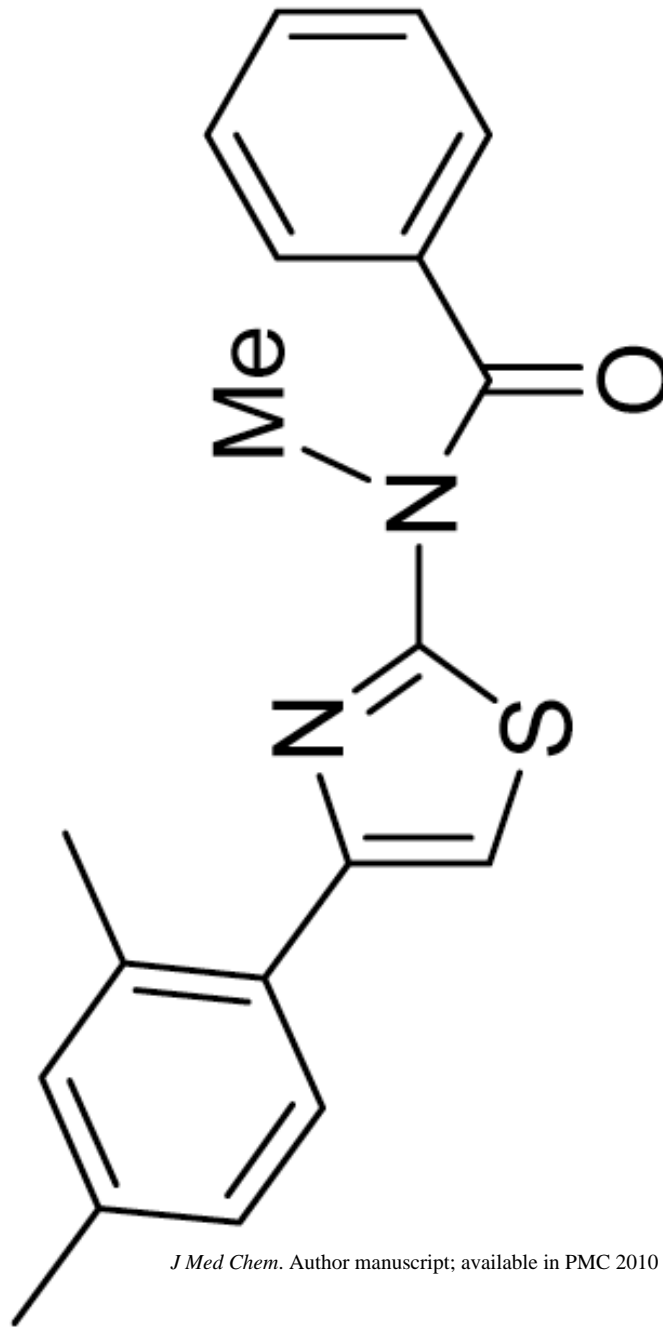
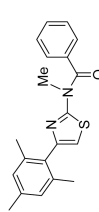
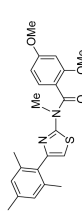
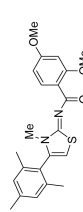
Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562

21		1.6	4.2	2.5	3.8
22		>40	>40	>40	>40

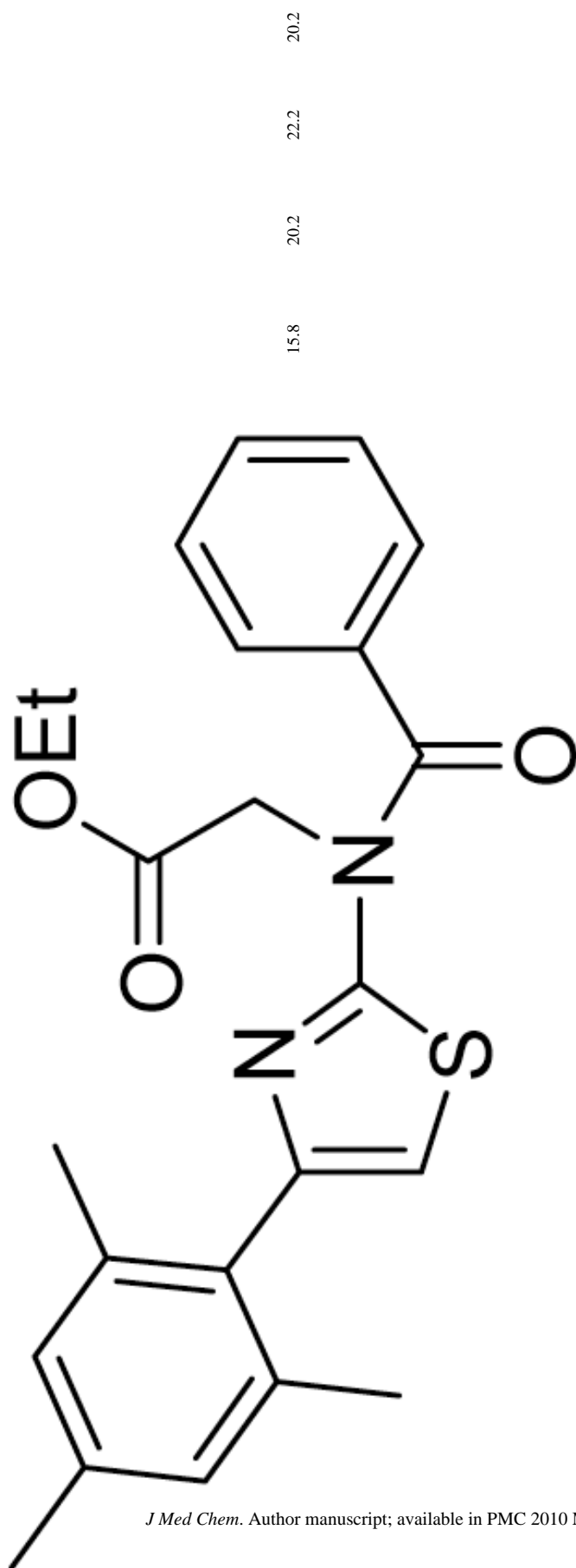
Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562



Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562

24		13.5	16.0	20.2	14.8
25		11.6	10.8	14.1	9.2
26		10.6	18.7	>40	15.5
27		22.1	21.0	22.0	23.7

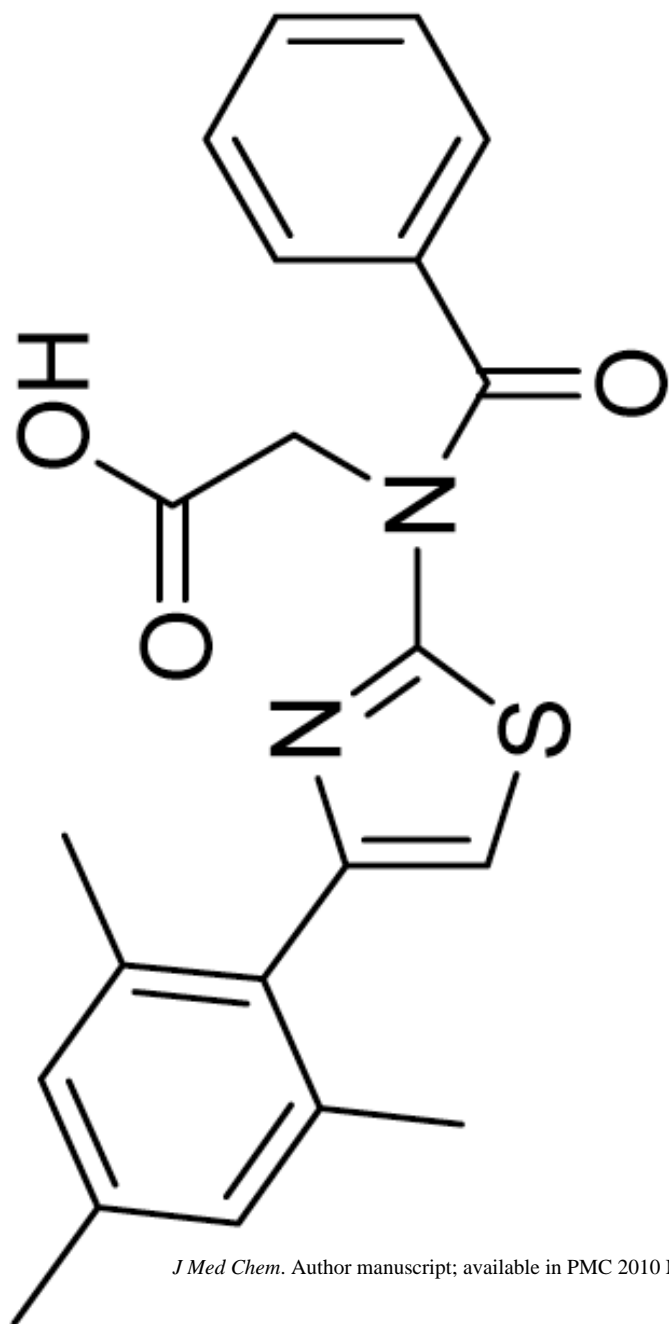
Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562



15.8 20.2 22.2 20.2

Compound	IC50 (μM)			
	MB231	MB468	HeLa	K562

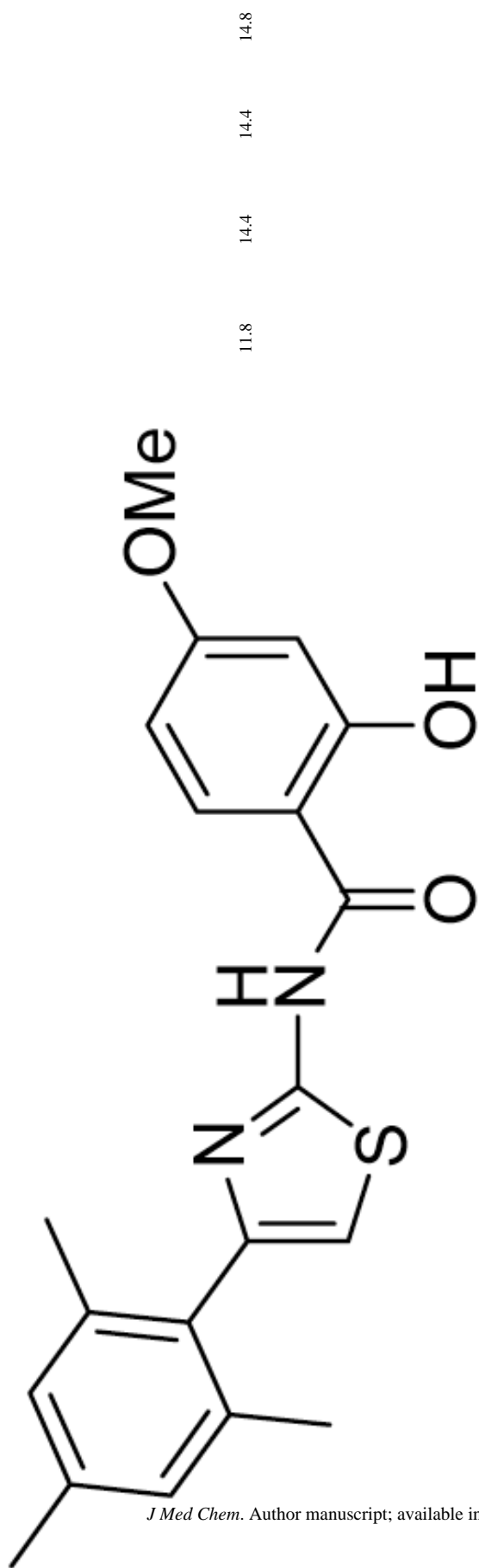
Structure



&gt;40 &gt;40 &gt;40 &gt;40



Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562

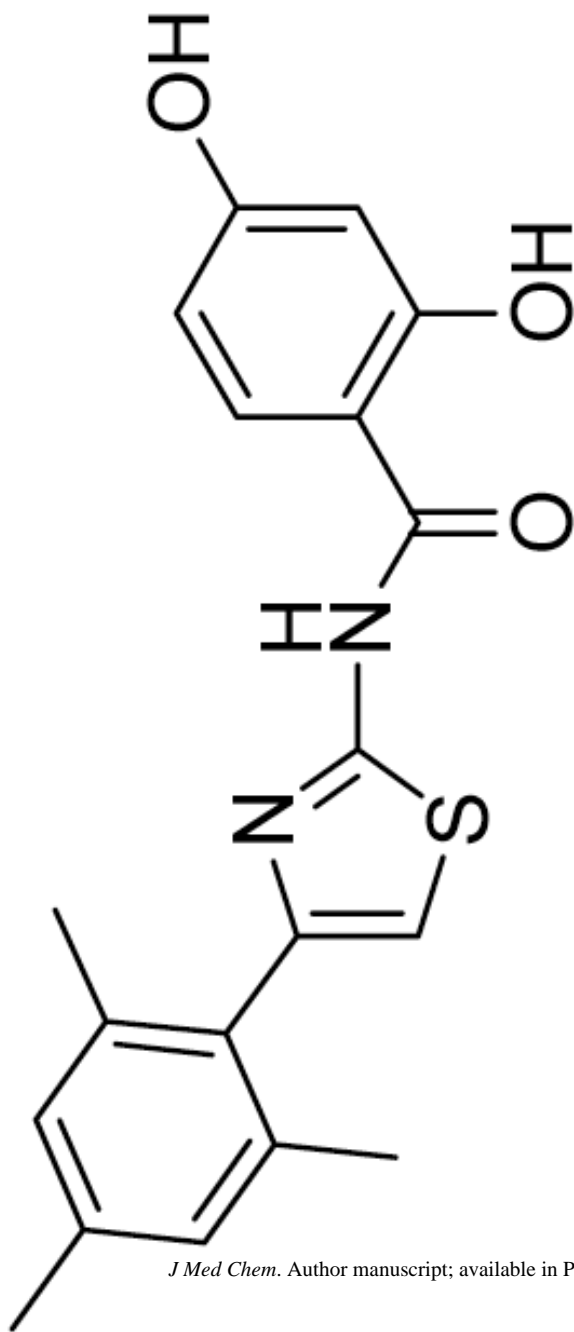


Compound

Structure

IC<sub>50</sub> (μM)

MB231 MB468 HeLa K562

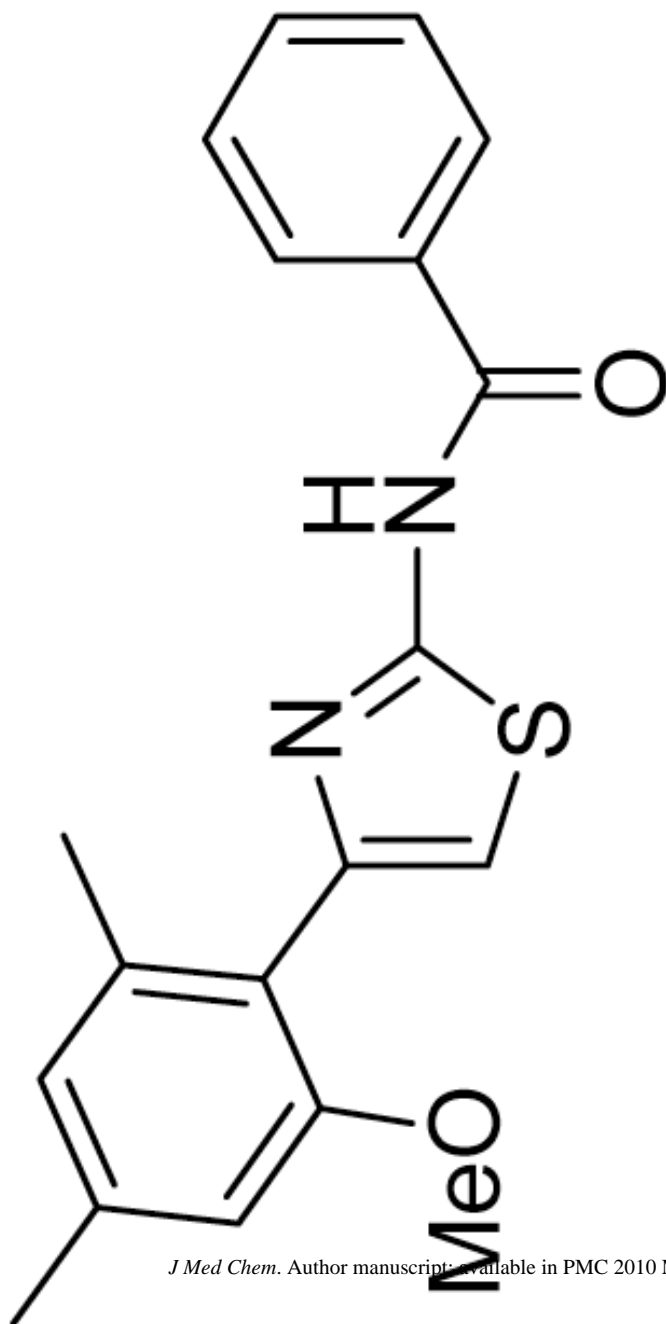


22.5 21.3 >40 >40

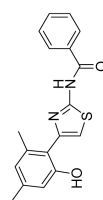
33

Compound	IC50 (μM)			
	MB231	MB468	HeLa	K562

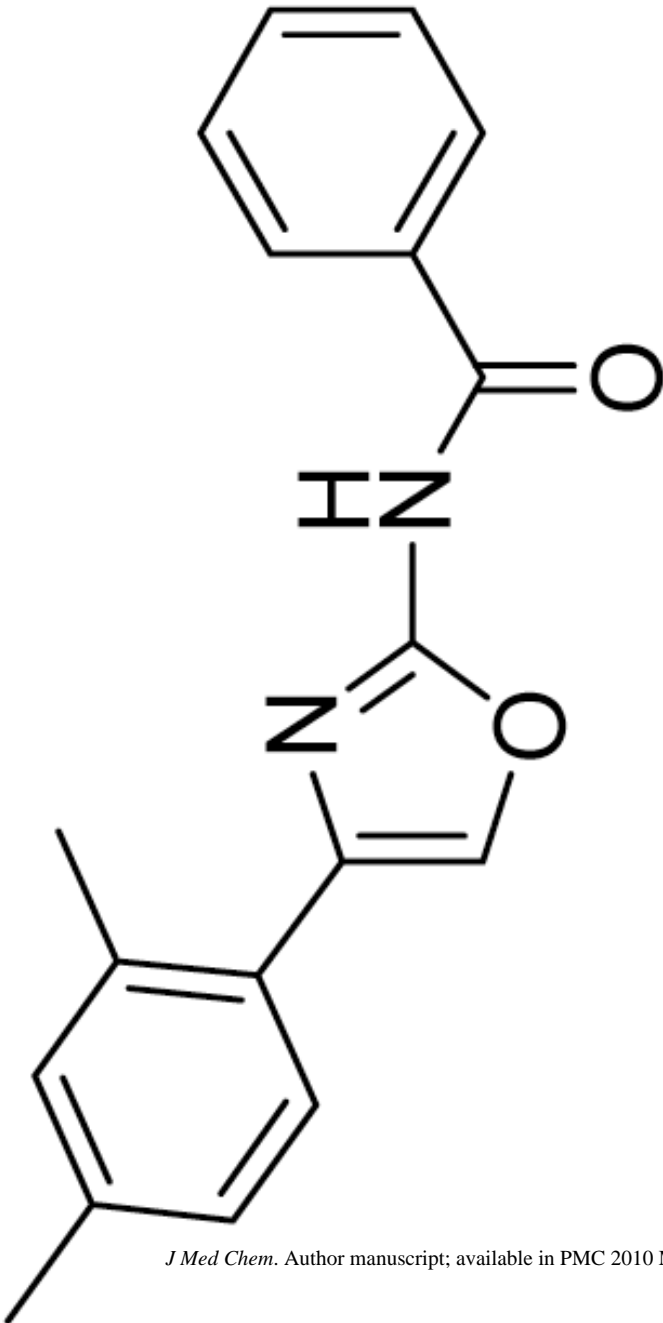
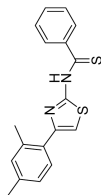
Structure



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Compound	Structure	IC50 (μM)			
		MB231	MB468	HeLa	K562
39		14.4	16.0	18.6	19.2
40		21.1	19.6	18.6	22.9