Design, Synthesis and SAR Studies of 4-Substitutedmethoxylbenzoyl-Aryl-Thiazoles Analogs as Potent and Orally Bioavailable Anticancer Agents

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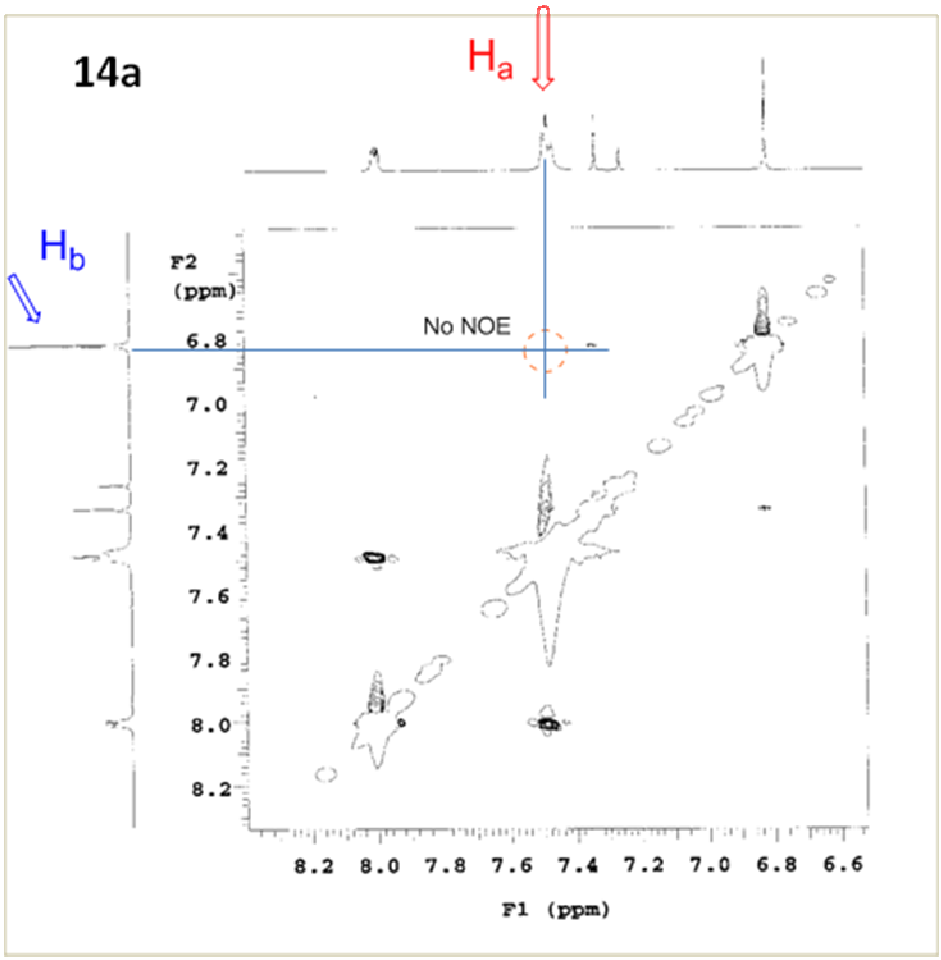
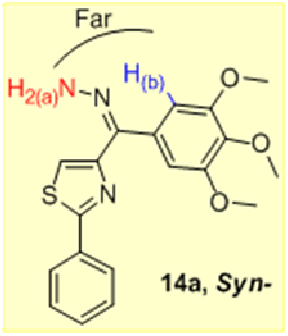
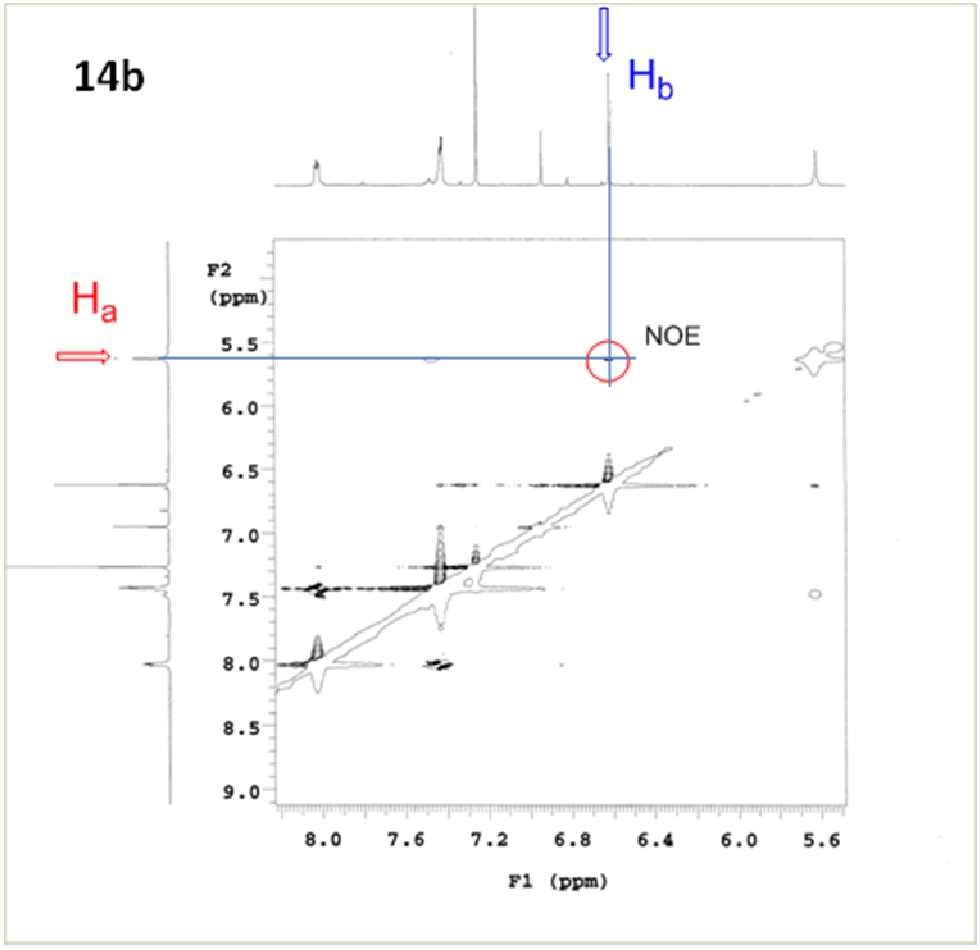
NOESY 2D of **14a** and **14b**



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(ppm)
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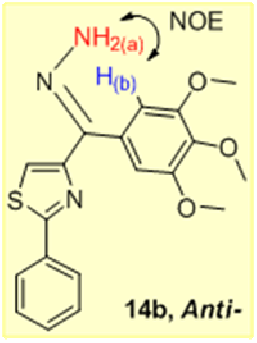
5.6
F1 (ppm)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$H_{(b)}$
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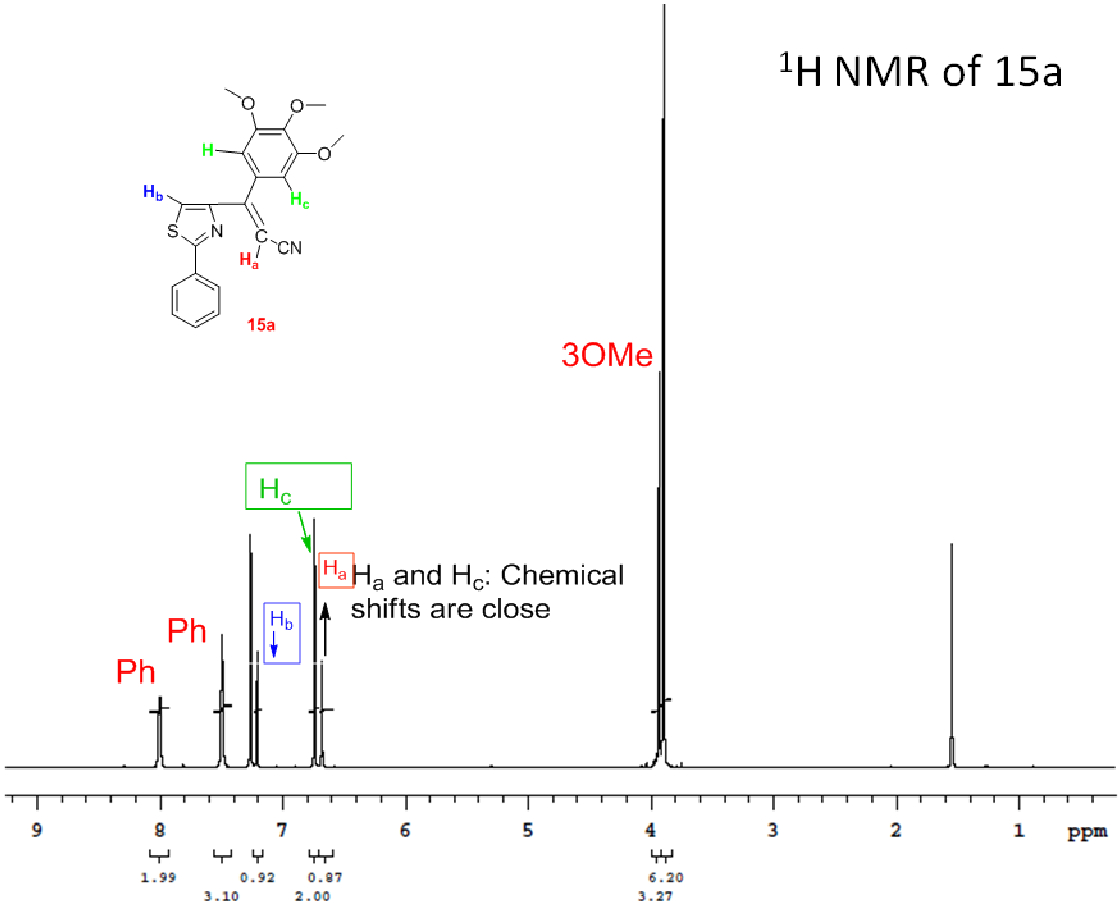
O
S
N
14b, *Anti-*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15a
3OMe
Ph
Ph
H_c
H_a
H_b
H_a and H_c: Chemical shifts are close
9
8
7
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3
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ppm
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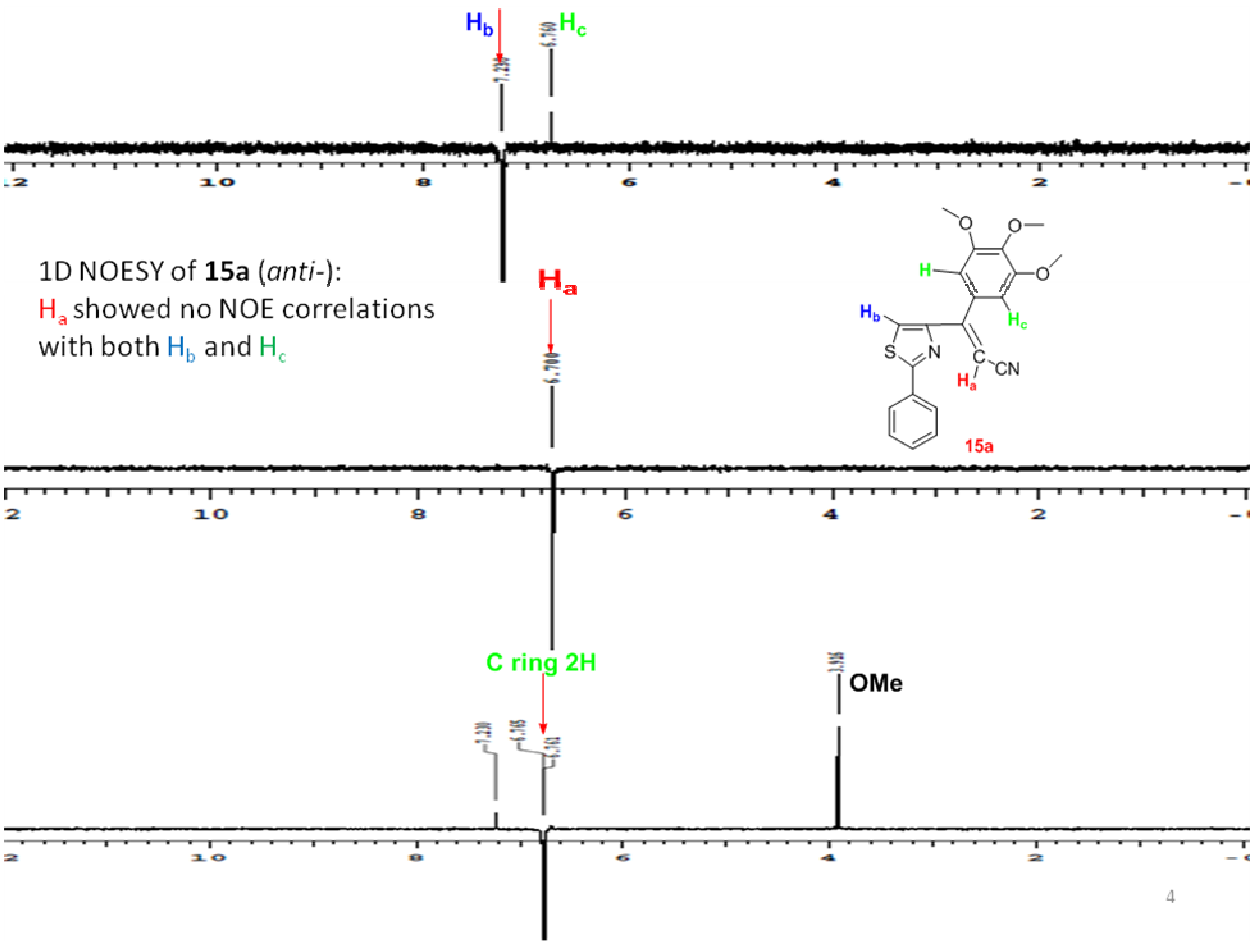
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H_c
1D NOESY of 15a (anti-):
H_a showed no NOE correlations
with both H_b and H_c
H_a
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O
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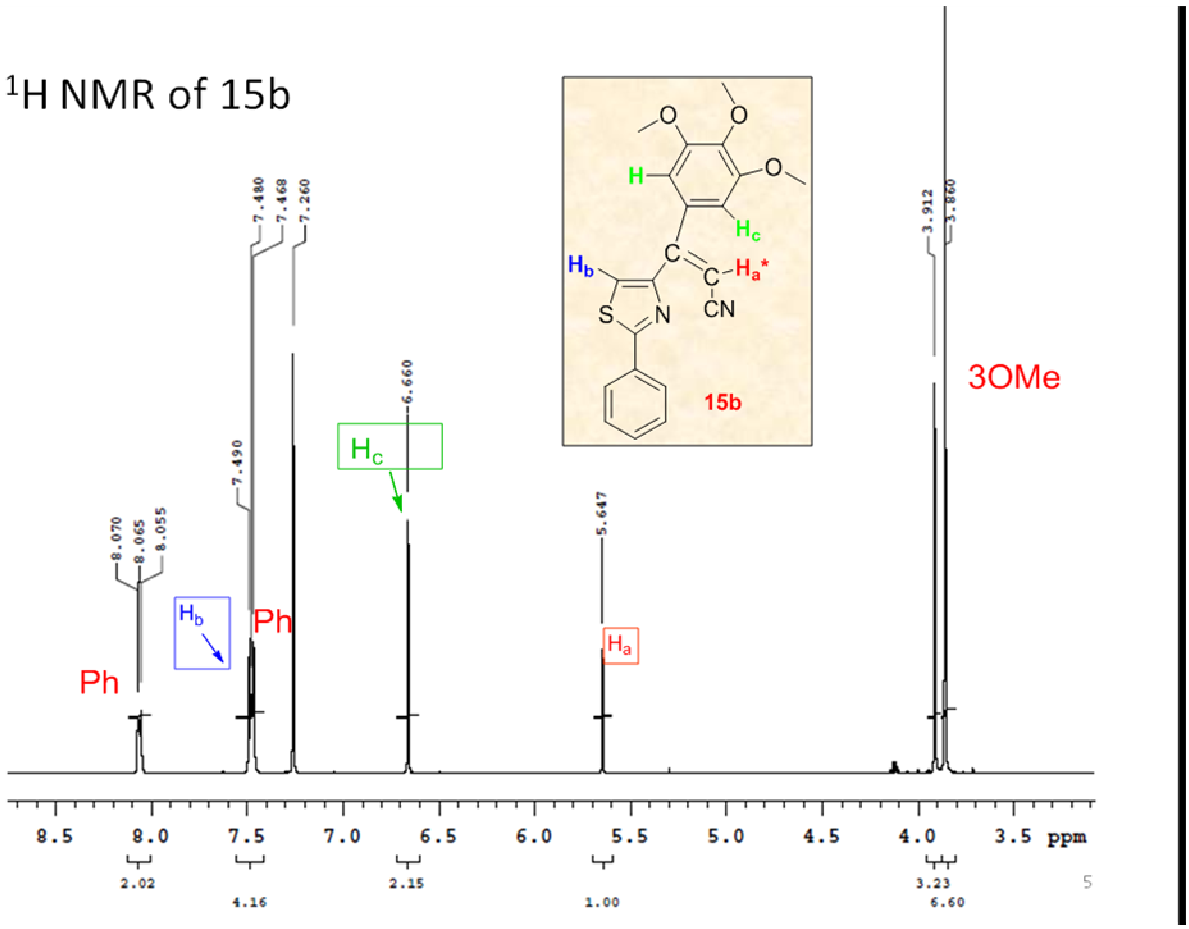
H_c
S
N
C
CN
H_a
15a
C ring 2H
OMe

4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3OMe
Ph
Ph
Hb
Hc
Ha
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3.860
8.5
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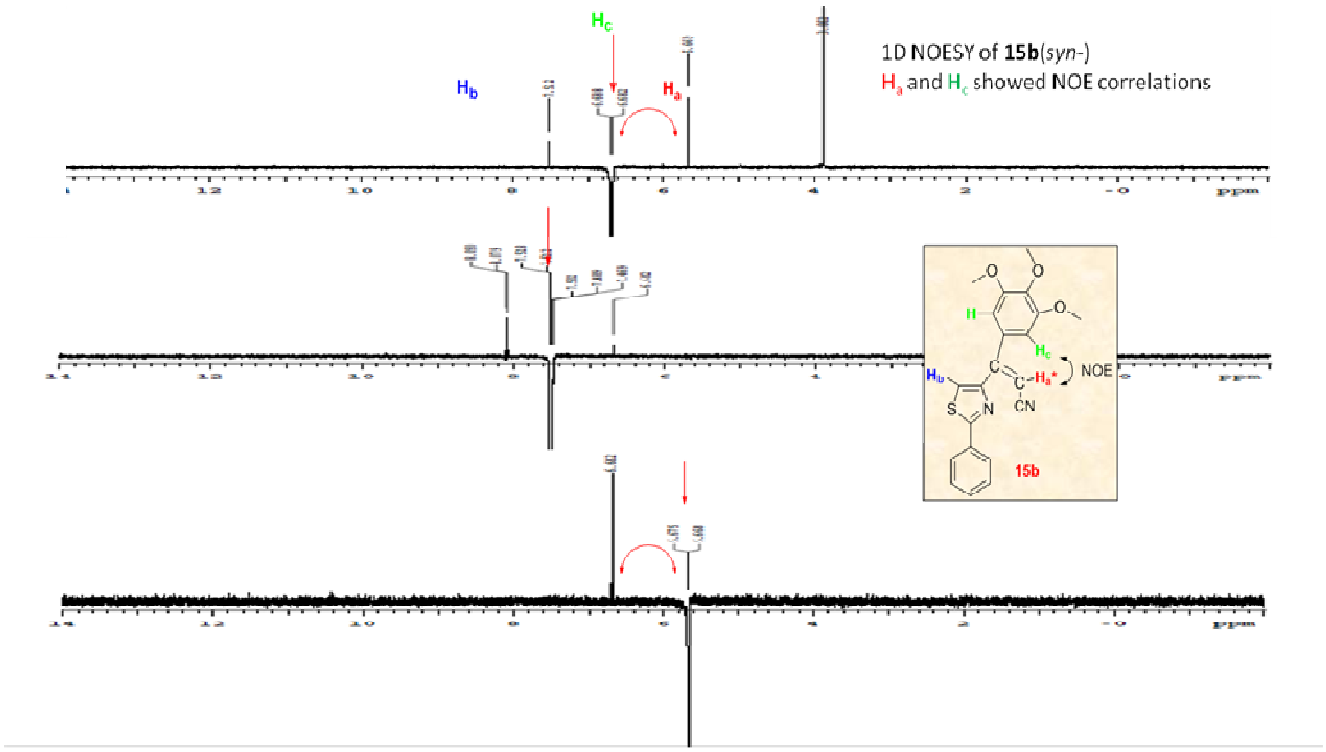

1D NOESY of 15b(syn-)
Ha and Hc showed NOE correlations
Hb
Hc
Ha
NOE
15b

***(2R)*-2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester 2.** Acetyl chloride (6.8 mL) was added dropwise to ice-cold methanol (30 mL). After the addition of L-serine (0.48 mmol), the reaction mixture was warmed to r.t. and stirred overnight. Evaporation of the solvent gave white solid *(2R)*-3-hydroxy-2-methyl-propionic acid methyl ester HCl salt, which was used without purification in the next step. Triethylamine (11 mL, 72.3 mmol) was added slowly to a solution of ethyl benzimidate hydrochloride (11.6 g, 62.8 mmol) in CH_2Cl_2 (150 mL). The reaction mixture was stirred at r. t. for 30 min and *(2R)*-3-hydroxy-2-methyl-propionic acid methyl ester HCl salt (13.5 g, 79.6 mmol) was added by portion. The resulting mixture was stirred for 48 h and concentrated under reduced pressure. The compound **2** was separated from flash column as yellow oil (12.3 g, 95.9%). ^{1}H NMR ($CDCl_3$) δ 7.99 -7.38 (m, 5 H), 4.97 (dd, 1 H, *J*= 7.8 Hz, *J*= 10.5 Hz), 4.70 (t, 1 H, *J*= 8.7 Hz), 4.62(dd, 1 H, *J*= 8.7 Hz, *J*= 10.5 Hz), 3.82 (s, 3 H); MS (ESI) m/z 206.1 $(M + H)^+$.

***(2R)*-2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid 3.** To an ice-cooled solution of **2** in MeOH/H_2O was added LiOH (2.5 equiv) with stirring. The mixture was allowed to warm to rt in 1 hour, concentrated in vacuo, and the white solid was dissolved in H_2O and acidified with 1 N HCl to pH 2.0 and extracted with $MgSO_4$, filtered and concentrated in vacuo to provide the acid **3** as a white solid (95.8 %). ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 2 H), 7.57-7.42 (m, 3 H), 5.04 (dd, 1 H, *J*= 7.8 Hz, *J*= 10.8 Hz), 4.80 (t, 1 H, *J*= 8.7 Hz), 4.70 (dd, 1 H, *J*= 9.0 Hz, *J*= 10.8 Hz); MS (ESI) m/z 191.9 $(M + H)^+$, 189.7 $(M - H)^-$, 145.8 $(M - COOH)^-$.

***(2R)*-2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methoxy-methyl-amide 4**. To a mixture of **3** (5 mmol), EDCI (6 mmol), HOBt (5 mmol) and Et_3N (5 mmol) in CH_2Cl_2 (50 mL) was added $HNCH_3OCH_3$ (5 mmol) and stirring continued at room temperature for 6-8 hours. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and sequentially washed with water, Satd.

$NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product **4**, which was purified by column chromatography as a white solid (61.0 %). ^{1}H NMR ($CDCl_3$) δ 7.98-7.36 (m, 5 H), 7.57-7.42 (m, 3 H), 5.35 (br, t, 1 H), 4.81 (br, t, 1 H), 4.52 (dd, 1 H, *J*= 8.7 Hz, *J*= 10.2 Hz), 3.90 (s, 3 H), 3.27 (s, 3 H); MS (ESI) m/z 257.0 $(M + H)^+$.

N-methoxy-N-methylbiphenyl-3-carboxamide 8a. To a mixture of biphenyl-3-carboxylic acid **7a** (5 mmol), EDCI (6 mmol), HOBt (5 mmol) and NMM (11 mmol) in CH_2Cl_2 (50 mL) was added $HNCH_3OCH_3HCl$ salt (5 mmol) and stirring continued at room temperature for 2 hours. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and sequentially washed with water, Satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a colorless oil, which was used for next step (58.4 %). MS (ESI) m/z 264.0 $(M + Na)^+$.

N-methoxy-N-methyl-6-phenylpyrimidine-4-carboxamide 8b. To a mixture of 6-phenylpyrimidine-4-carboxylic acid **7b** (5 mmol), EDCI (6 mmol), HOBt (5 mmol) and NMM (11 mmol) in CH_2Cl_2 (50 mL) was added $HNCH_3OCH_3HCl$ salt (5 mmol) and stirring continued at room temperature for overnight. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and sequentially washed with water, Satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8b** as a yellow solid (62.3 %). ^{1}H NMR ($CDCl_3$) δ 9.28 (s, 1 H), 8.14-8.06 (m, 2 H), 7.96 (br, s, 1 H), 7.54-7.50 (m, 3 H), 5.35 (br, t, 1 H), 4.81 (br, t, 1 H), 4.52 (dd, 1 H, *J*= 8.7 Hz, *J*= 10.2 Hz), 3.79 (s, 3 H), 3.42 (s, 3 H); MS (ESI) m/z 266.0 $(M + Na)^+$.

N-methoxy-N-methyl-6-phenylpicolinamide 8c. To a mixture of 6-phenylpicolinic acid **7c** (1.77 mmol), EDCI (2.12 mmol), HOBt (1.86 mmol) and NMM (3.54 mmol) in CH_2Cl_2 (20 mL) was added $HNCH_3OCH_3HCl$ salt (1.86 mmol) and stirring continued at room temperature for overnight. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and sequentially washed with water, Satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8c** as a colorless oil (51.2 %). ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1 H, *J*= 7.0 Hz), 7.86-7.81 (m, 2 H), 7.55 (br, 1 H), 7.48 (t, 2 H), 7.44-7.41 (m, 1 H), 3.82 (s, 3 H), 3.44 (s, br, 3 H); MS (ESI) m/z 265.0 $(M + Na)^+$.

N-methoxy-N-methyl-5-phenylfuran-2-carboxamide 8d. To a mixture of 5-phenylfuran-2-carboxylic acid **7d** (10 mmol), EDCI (12 mmol), HOBt (11 mmol) and NMM (21 mmol) in CH_2Cl_2 (200 mL) was added $HNCH_3OCH_3HCl$ salt (10.5 mmol) and stirring continued at room temperature for overnight. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and sequentially washed with water, Satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8d**. (95.2 %). ^{1}H NMR ($CDCl_3$) δ 7.82 (d, 1 H, *J*= 7.0 Hz), 7.46-7.43 (t, 2 H), 7.37-7.34 (m, 1 H), 7.25 (d, 1 H, *J*= 4.0 Hz), 6.78 (d, 1 H, *J*= 4.0 Hz), 3.86 (s, 3 H), 3.41 (s, 3 H); MS (ESI) m/z 254.1 $(M + Na)^+$.

N-methoxy-N-methyl-5-phenylthiophene-3-carboxamide 8e. To a mixture of 5-phenylthiophene-3-carboxylic acid **7e** (2.5 mmol), EDCI (2.9 mmol), HOBt (2.6 mmol) and NMM (5.3 mmol) in CH_2Cl_2 (30 mL) was added $HNCH_3OCH_3HCl$ salt (2.6 mmol) and stirring continued at room temperature for overnight. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and sequentially washed with water, Satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The

solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8e**. (90.8 %). ^{1}H NMR ($CDCl_3$) δ 8.28 (d, 1 H, *J*= 1.5 Hz), 7.69 (d, 1 H, *J*= 1.5 Hz), 7.64 (d, 2 H, *J*= 7.0 Hz), 7.44 (t, 2 H, *J*= 7.0 Hz), 7.35-7.32 (m, 1 H), 6.78 (d, 1 H, *J*= 4.0 Hz), 3.86 (s, 3 H), 3.41 (s, 3 H); MS (ESI) m/z 270.0 (M + Na)$^+$.

N-methoxy-N-methyl-5-phenylthiophene-3-carboxamide 8f. To a mixture of 3-phenyl-1H-pyrazole-5-carboxylic acid **(7f)** (5.3 mmol), EDCI (6.4 mmol), HOBt (5.85 mmol) and NMM (12.9 mmol) in CH_2Cl_2 (50 mL) was added $HNCH_3OCH_3HCl$ salt (5.85 mmol) and stirring continued at room temperature for overnight. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and sequentially washed with water, Satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8f**. (75.3 %). ^{1}H NMR ($CDCl_3$) δ 11.3 (br, 1 H), 7.84 (d, 2 H, *J*= 7.0 Hz), 7.43 (t, 2 H, *J*= 7.5 Hz), 7.35 (t, 1 H, *J*= 7.5 Hz), 7.12 (s, 1 H), 3.83 (s, 3 H), 3.42 (s, 3 H); MS (ESI) m/z 254.1 (M + Na)$^+$.

(5-Phenylthiophen-3-yl)(3,4,5-trimethoxyphenyl)methanol 10e. At -78 ^{o}C, to a solution of **8e** (2.5 mmol) in 5 mL THF under argon protection was added a solution of $LiAlH_4$ in THF (1 N, 1.42 mL) and stirring continued at 1 hour at -20^{o}C. The reaction mixture was placed on an ice bath and quenched by 20% H_2SO_4 solution, extracted with ethyl acetate and dried over $MgSO_4$. The solvent was removed under reduced pressure and purified by column chromatography to yield 5-phenylthiophene-3-carbaldehyde (84.8%). ^{1}H NMR ($CDCl_3$) δ 9.98 (s, 1 H), 8.04 (d, 1 H, *J*= 1.5 Hz), 7.86 (br, 1 H), 7.61-7.58 (br, 2 H), 7.47-7.33 (m, 3 H), 7.35-7.32 (m, 1 H), 6.78 (d, 1 H, *J*= 4.0 Hz); MS (ESI) m/z 210.9 (M + Na)$^+$. To a solution of 5-phenylthiophene-3-carbaldehyde (0.195g, 1.04 mmoL) in 5 mL THF was added a THF solution of 3, 4, 5-

trimethoxyphenylmagnesiumbromide (0.5 N, 2.3 mL, 1.14 mmol) at 0 ^{o}C. The mixture was allowed to stirring for 30 min and quenched with satd. NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **10e**. (70.5%).^{1}H NMR ($CDCl_3$) δ 7.55-7.52 (m, 2 H), 7.40-7.35 (m, 3 H), 7.30 (br, 1 H), 7.20 (br, 1 H), 6.72 (s, 2 H), 6.01 (d, 1 H, *J*= 3.9 Hz), 3.86 (s, 6 H), 3.85 (s, 3 H), 2.42 (d, 1 H, *J*= 3.9 Hz); MS (ESI) m/z 339.1 $(M - OH)^{-}$.

(2-Phenylthiazol-5-yl)(3,4,5-trimethoxyphenyl)methanol 10g. To a solution of 2-phenylthiazole-5-carbaldehyde **8g** (0.567g, 3 mmoL) in 15 mL THF was added a THF solution of 3, 4, 5-trimethoxyphenylmagnesiumbromide (0.5 N, 6.5 mL, 3.25 mmol) at 0 ^{o}C. The mixture was allowed to stirring for 30 min and quenched with satd. NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **10g** (72.9 %).^{1}H NMR ($CDCl_3$) δ 7.90 (m, 2 H), 7.64 (s, 1 H), 7.41 (m, 3 H), 6.69 (s, br, 2 H), 6.04 (s, 1 H), 3.86 (s, 6 H), 3.85 (s, 3 H), 1.57 (d, 1 H, *J*= 5.5 Hz); MS (ESI) m/z 358.1 $(M + Na)^{+}$.

(5-Phenylisoxazol-3-yl)(3,4,5-trimethoxyphenyl)methanol 10h. To a solution of 5-phenylisoxazole-3-carbaldehyde **8h** (0.365g, 2.1 mmoL) in 15 mL THF was added a THF solution of 3, 4, 5-trimethoxyphenylmagnesiumbromide (0.5 N, 5.5 mL, 2.74 mmol) at 0 ^{o}C. The mixture was allowed to stirring for 30 min and quenched with satd. NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **10h** as a white solid. (48.8%). ^{1}H NMR ($CDCl_3$) δ 7.78-7.77 (m, 2 H), 7.48-7.46 (m, 3 H), 6.74 (s, 2 H), 6.45 (s,

1 H), 5.98 (d, 1 H, *J*= 3.5 Hz) 3.89 (s, 6 H), 3.86 (s, 3 H), 2.77 (d, 1 H, *J*= 3.5 Hz); MS (ESI) m/z 364.1 $(M + Na)^+$.

2-Phenyl-4, 5-dihydrothiazole-4-carboxylic acid 12a. Benzonitrile (40 mmol) was combined with *L*- Cysteine (45mmol) in 100 mL of 1:1 MeOH/pH6.4 phosphate buffer solution. The reaction was stirred at 40 ^oC for 3 days. The precipitate was removed by filtration, and MeOH was removed using rotary evaporation. The remaining solution was added 1M HCl to adjust pH = 2 under 0 ^oC. The resulting precipitate was filtered to yield a white solid 2-phenyl-4, 5-dihydrothiazole-4-carboxylic acid **12a**, which was used directly to next step without purification.

(2-Phenyl-thiazol-4-yl)-(3,4,5-trimethoxy-phenyl)-methanone 1. A mixture of 2-phenyl-4, 5-dihydrothiazole-4-carboxylic acid (5 mmol), EDCI (6 mmol) and HOBt (5 mmol) in CH_2Cl_2 (50 mL) was stirred for 10 min. To this solution, NMM (5 mmol) and $HNCH_3OCH_3$ (5 mmol) were added and stirring continued at room temperature for 6-8 hours. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and sequentially washed with water, satd. $NaHCO_3$, Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to get 2-phenyl-4, 5-dihydrothiazole-4-carboxylic acid methoxymethylamide. A solution of 2-phenyl-4, 5-dihydrothiazole-4-carboxylic acid methoxymethylamide (1 equiv) in CH_2Cl_2 was cooled to 0^oC, and distilled DBU (2 equiv) was added. Bromotrichloromethane (1.7 equiv) was then introduced dropwise via syringe over 10 min. The reaction mixtures were allowed to warm to room temperature and stirred overnight. Upon washing with satd. aqueous NH_4Cl (2 × 50 mL), the aqueous phase was extracted with EtOAc (3× 50 mL). The combined organic layers were dried on $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography as needed providing compounds 2-phenyl-thiazole-4-carboxylic acid methoxymethylamide (73.6 %). ^1H NMR

(300MHz, $CDCl_3$) δ 8.01 (s, 1 H), 7.99-7.96 (m, 2 H), 7.47-7.44 (m, 3 H), 3.88 (s, 3 H), 3.49 (s, 3 H). MS (ESI) *m/z* 271.0 $(M + Na)^+$. To a solution of 3, 4, 5-trimethoxyphenylmagnesium bromide (0.5 N, 3 mL) in 2 mL THF was charged a solution of 2-phenyl-thiazole-4-carboxylic acid methoxymethylamide (1 mmol) in 3 mL THF at 0 ^oC. The mixtures were stirred for 30 min until amides disappeared on TLC plates. The reaction mixture was quenched with satd. NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **1**. Yield: 27.3 %. ^1H NMR (300 MHz, $CDCl_3$): δ 8.29 (s, 1 H), 8.03 (q, 2 H), 7.80 (s, 2 H), 7.49-7.47 (m, 3 H), 3.96 (s, 6 H), 3.97 (s, 3 H). MS (ESI) *m/z* 378.1 $(M + Na)^+$.

2-Phenylthiazole-4-carbaldehyde 12b. At -78^oC, to a solution of 2-phenyl-thiazole-4-carboxylic acid methoxymethylamide (1equiv) in THF was added $LiAlH_4$ (1 equiv, 1 N in THF) and stirring for 1 hour at -20^oC. The reaction mixture was placed on an ice bath and quenched by 20% H_2SO_4 solution, extracted with ethyl acetate and dried over $MgSO_4$. The solvent was removed under reduced pressure and purified by column chromatography to yield **12b** (45.8 %). ^1H NMR (300 MHz, $CDCl_3$): δ 10.1 (s, 1 H), 8.17 (s, 1 H), 8.02-8.00 (m, 2 H), 7.50-7.48 (m, 3 H). MS (ESI) *m/z* 244.1 $(M + Na + MeOH)^+$.

(*R*)-2-(4-Hydroxyphenyl)-N-methoxy-N-methyl-4,5-dihydrothiazole-4-carboxamide 27 was synthesized using the same method as used of 8d. Quatitative yield. ^1H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, 2 H, *J* = 8.5 Hz), 6.84 (br, 1 H), 6.73 (d, 2 H, *J* = 8.5 Hz), 5.64 (t, br, 1 H), 3.87 (s, 3 H), 3.30 (s, 3 H). MS (ESI) *m/z* 289.0 $(M + Na)^+$, 264.9 $(M -H)^-$.

1H-indole-2-carbonitrile 30. To a cooled solution of indole-2-carboxylic acid (2.0 g, 12.4 mmol) in 60 mL of anhydrous Et_2O was added 1.9 mL of $SOCl_2$ (26 mmol). After stirring for 40 min at room temperature, the ether was removed under reduced pressure at a temperature not

exceeding 35 ^{o}C. The obtained acyl chloride was dissolved in 40 mL of anhydrous Et_2O and the resulting solution was added immediately to a stirred solution of liquid ammonia in 80 ml of Et_2O. The reaction mixture was stirred at room temperature for 24 h. The solvent was then evaporated under reduced pressure, and the white indole-2-carboxamide was crystallized from 50% aq EtOH and dried in air, after which it was dissolved in $POCl_3$ and heated under reflux for 5 min. The cooled solution was poured onto crushed ice and aq NH4OH was added to maintain a basic pH. The aqueous mixture was extracted with Et_2O, the extracts were dried over Na_2SO_4 and evaporated. The brown indole-2-carbonitrile (63.3% overall yield from indole-2-carboxylic acid) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (br, s, 1 H), 7.68 (d, 1 H, *J* = 8.0 Hz), 7.43-7.34 (m, 2 H), 7.24-7.21 (m, 2 H). MS (ESI) *m/z* 144.0 $(M + H)^+$, 140.8 $(M -H)^-$.

(R)-2-(1H-indol-2-yl)-N-methoxy-N-methyl-4,5-dihydrothiazole-4-carboxamide 31 was synthesized using the same method as used of 8d. 67.1% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.06 (s, br, 1 H), 7.64 (d, 2 H, *J* = 8.1 Hz), 7.36-7.24 (m, 2 H), 7.12 (dt, 1 H, *J* = 8.1 Hz, 1.2 Hz), 6.95 (d, 1 H, *J* = 1.8 Hz), 5.60 (t, br, 1 H, *J* = 8.7 Hz), 3.86 (s, 3 H), 3.78 (t, 1 H, *J* = 10.2 Hz), 3.58 (dd, 1 H, *J* = 9.0 Hz, 10.2 Hz), 3.30 (s, 3 H). MS (ESI) *m/z* 312.1 $(M + Na)^+$, 287.9 $(M -H)^-$.

(R)-2-(1-(Phenylsulfonyl)-1H-indol-5-yl)-4,5-dihydrothiazole-4-carboxylic acid 34. (R)-2-(1H-indol-5-yl)-4,5-dihydrothiazole-4-carboxylic acid **33** was synthesized using the same method as used of **12a** from 1H-indole-5-carbonitrile and used without further purification. To a vigorously stirring solution of **33** (1 mmol) and tetrabutylammonium hydrogen sulfate (0.15 mmol) in toluene (10 mL) at 0 °C was added 50% aqueous sodium hydroxide (10 mL) and sulfonyl chloride (2 mmol). The resultant solution was stirred at room temperature for 6 h. Then 1 N HCl was added to acidify the mixture to pH=2 and extracted with CH_2Cl_2, the organic layer

was separated and dried (MgSO4); then evaporated to dryness to yield **34**, which were used in subsequent steps without further purification.

(R)-N-methoxy-N-methyl-2-(1-(phenylsulfonyl)-1H-indol-5-yl)-4,5-dihydrothiazole -4-carboxamide 35 was prepared with the same method as used of 8d from 34. 57.1% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.92 (m, 2 H), 7.77 (m, 3 H), 7.51 (d, 1 H, *J* = 3.0 Hz), 7.46 (t, 1 H), 7.35 (t, 1H), 6.61 (d, 1 H), 5.58 (br, t, 1 H) 3.82 (s, 3 H), 3.73 (t, 1 H), 3.43 (m, 1 H), 3.21 (s, 3 H). MS (ESI) *m/z* 452.1 $(M + Na)^+$.

(R)-*tert*-Butyl 4-(4-(methoxy(methyl)carbamoyl)-4,5-dihydrothiazol-2-yl)benzyl carbamate 37. 4-(aminomethyl)benzonitrile (25.09 g, 0.149 mol) and L-cysteine (18.1 g, 0.149 mol) were suspended in 500 mL MeOH and pH 6.4 buffer solutions (1:1) and stirred for 3 days at room temperature. Triethylamine (30 mL) was added to the mixture and Boc_2O (68 g, 0.31mol) was added to this mixture and stirred for 2 hours. The solvents were removed and filtered to yield white solid (*R*)-2-(4-((tert-butoxycarbonylamino)methyl)phenyl)-4,5-dihydrothiazole-4-carboxylic acid (38.4 g, 76.8%). Compound **37** was obtained following the same method as used of **8d** from this acid. Yield: 84.4 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 - 7.77 (d, 2 H, *J* = 7.5 Hz), 7.27 - 7.26 (d, 2 H, *J* = 7.5 Hz), 7.23 (s, 1 H), 5.62 (br, 1 H), 4.87 (br, 1 H), 4.30 (br, 2 H), 3.86 (s, 3 H), 3.78 (t, *J* = 10.0 Hz, 1 H), 3.48 - 3.4 (m, 1 H), 3.25 (s, 3 H), 1.42 (s, 9 H). MS (ESI) m/z 402.1$(M + Na)^+$, 378.0 $(M - H)^-$.

***tert*-Butyl 4-(4-(3,4,5-trimethoxybenzoyl)thiazol-2-yl)benzylcarbamate 38.** To a solution of (3,4,5-trimethoxyphenyl)magnesium bromide (0.5 M, 5.5 mL) in THF was added a solution of 37 (1.83 mmol) in 10 mL THF under 0 ^oC and stirred for 30 mins. The reaction mixture was quenched with satd. NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column

chromatography to obtain pure compound as a light yellow solid (32.3 %). ^{1}H NMR (300M, $CDCl_3$) δ 8.27 (s, 1 H), 7.98 (d, 2 H, *J* = 8.1 Hz), 7.78 (s, 2 H), 7.39 (d, 2 H, *J* = 8.1 Hz), 7.27 - 7.26 (d, 2 H, *J* = 7.5 Hz), 7.23 (s, 1 H), 4.93 (br, 1 H), 4.37 (br, d, 1 H), 3.96 (s, 3 H), 3.95 (s, 6 H), 1.47 (s, 9 H).MS (ESI) m/z 507.1$(M + Na)^+$; Anal. ($C_{25}H_{28}N_2O_6S$) C, H, N.

***tert*-Butyl methyl(4-(4-(3,4,5-trimethoxybenzoyl)thiazol-2-yl)benzyl)carbamate (41).** At 0 ^oC, to a solution of compound **38** (100 mg, 0.2 mmol) in 5 mL DMF was added sodium hydride (10 mg, 0.2 mmol), then iodomethane (77 mg, 0.4 mmol) was added to the reaction mixture and stirred at RT overnight. The mixture was quenched with a sat. $NaHCO_3$ solution, extracted with ethyl acetate and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **41**. Yield: 61.3%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (s, 1 H), 8.02 (d, 2 H, *J* = 8.0 Hz), 7.82 (s, 2 H), 7.36 (br, 2 H), 4.50 (s, 2 H), 4.00 (s, 3 H), 3.98 (s, 6 H), 2.90 (d, br, 3 H), 1.50 (s, 9 H). MS (ESI) *m/z* 521.2 $(M + Na)^+$, 496.9 $(M – H)^-$; Anal. ($C_{26}H_{30}N_2O_6S$) C, H, N.

General procedure for the synthesis of 2-(arylamino) thiazole-4-carboxylic acid 43a-c. N-Aryl thiourea (0.01 mol) and ethyl bromopyruvate (0.011 mol) were dissolved in 3 mL ethanol and held at reflux for 2 hrs. The reaction was cooled, the crystalline ethyl 2-(substituted phenylamino) thiazole-4-carboxylates were collected by filtration and washed with ethanol. Refluxing the mixture of ethyl esters with the NaOH-ethanol solution gave final compounds **43a-c** which were used directly in next steps.

N-methoxy-N-methyl-2-(arylamino)thiazole-4-carboxamide 44a-c were synthesized using the same method as used of 8d.

N-methoxy-N-methyl-2-(phenylamino)thiazole-4-carboxamide 44a. 90.2% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (s, 2 H), 7.38 (br, 1 H), 7.36-7.33 (m, br, 4 H), 7.09 (t, br, 1 H), 3.77 (s, 3 H), 3.43 (s, 3 H), 2.33 (s, 3 H). MS (ESI) *m/z* 286.0 $(M + Na)^+$.

N-methoxy-N-methyl-2-(p-tolylamino)thiazole-4-carboxamide 44b. 93.3% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (s, 1 H), 7.31 (br, 1 H), 7.22 (d, 2 H), 7.16 (d, 2 H), 3.76 (s, 3 H), 3.42 (s, 3 H), 2.33 (s, 3 H). MS (ESI) *m/z* 278.0 $(M + H)^+$.

2-(4-Fluorophenylamino)-N-methoxy-N-methylthiazole-4-carboxamide 44c. 89.7% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 (s, 1 H), 7.36-7.31 (m, 2 H), 7.07-7.04 (m, 6 H), 3.76 (s, 3 H), 3.42 (s, 3 H). MS (ESI) *m/z* 282.0 $(M + Na)^+$, 280.8 $(M - H)^-$.

NMR shift calculation methods:

All theoretical calculations were performed using Schrodinger Molecular Modeling Suite (Schrodinger, LLC, New York, NY) on a Dell Linux workstation. Both isomers were built and their energies were minimized using OPLS_2005 forcefield until gradient converged at 0.05 kJ/mol. In order to provide a good starting point for the much more expensive quantum mechanical calculations, we first performed a forcefield based conformational search to identify the global minimum. Default parameters were used except we chose systematic torsional sampling as the searching method and 5000 step conformational search for each isomer. Two distinct, symmetrical conformers were found for each of the isomers, with different orientation of the thiazole "B" ring relative to the trimethoxyphenyl "C" ring. These four isomers were used as starting conformation for subsequent geometry optimizations with density functional theory (DFT).

DFT calculations were performed with Jugar module within Schrodinger software. The basis set were 6-31G** with five D functions. The functionals in DFT were set to hygrid B3LYP. Solvent was set to DMSO, the same solvent for our NMR experiments. Geometry optimizations were performed before NMR shielding constants were calculated for the four structures. NMR shielding constants for tetramethyl silane (TMS) were calculated in the same way as the four isomers, and the NMR shielding constants relative to TMS were calculated to provide theoretical chemical shifts of the isomers in DMSO solution.

Results:

The optimized geometries for each of the two isomers were shown in Figure 1.

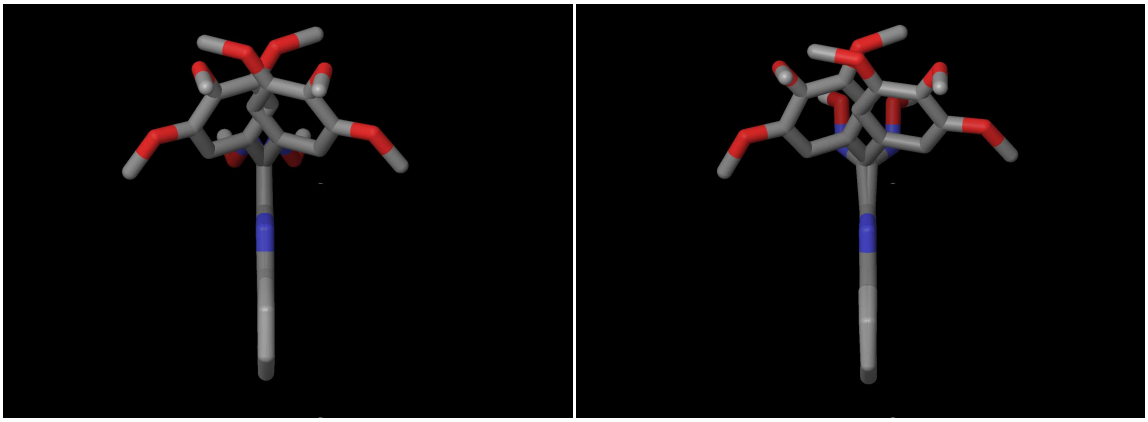


Figure 1. Two lowest energy conformers for each of the two isomers (left: isomer 1(**13a**); right: isomer 2(**13b**))

The dihedral angles for C-ring-carbon-->CN-carbon-->B-ring-carbon-->B-ring-nitrogen are +/- 22 degree for isomer 1, and +/- 34 degree for isomer 2. The average of the chemical shifts for the two conformers were used to compare experimental chemical shifts obtained in DMSO and is shown in Figure 2.

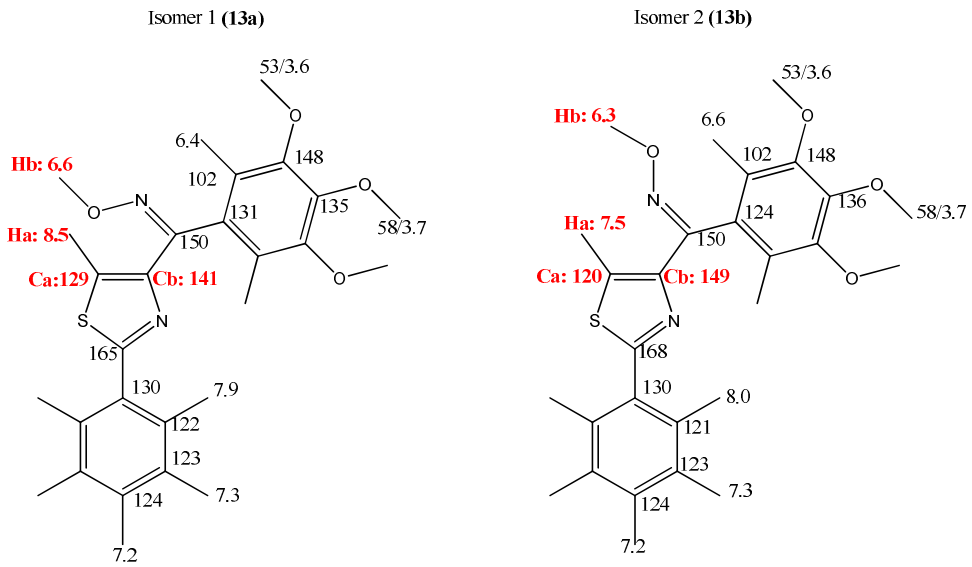


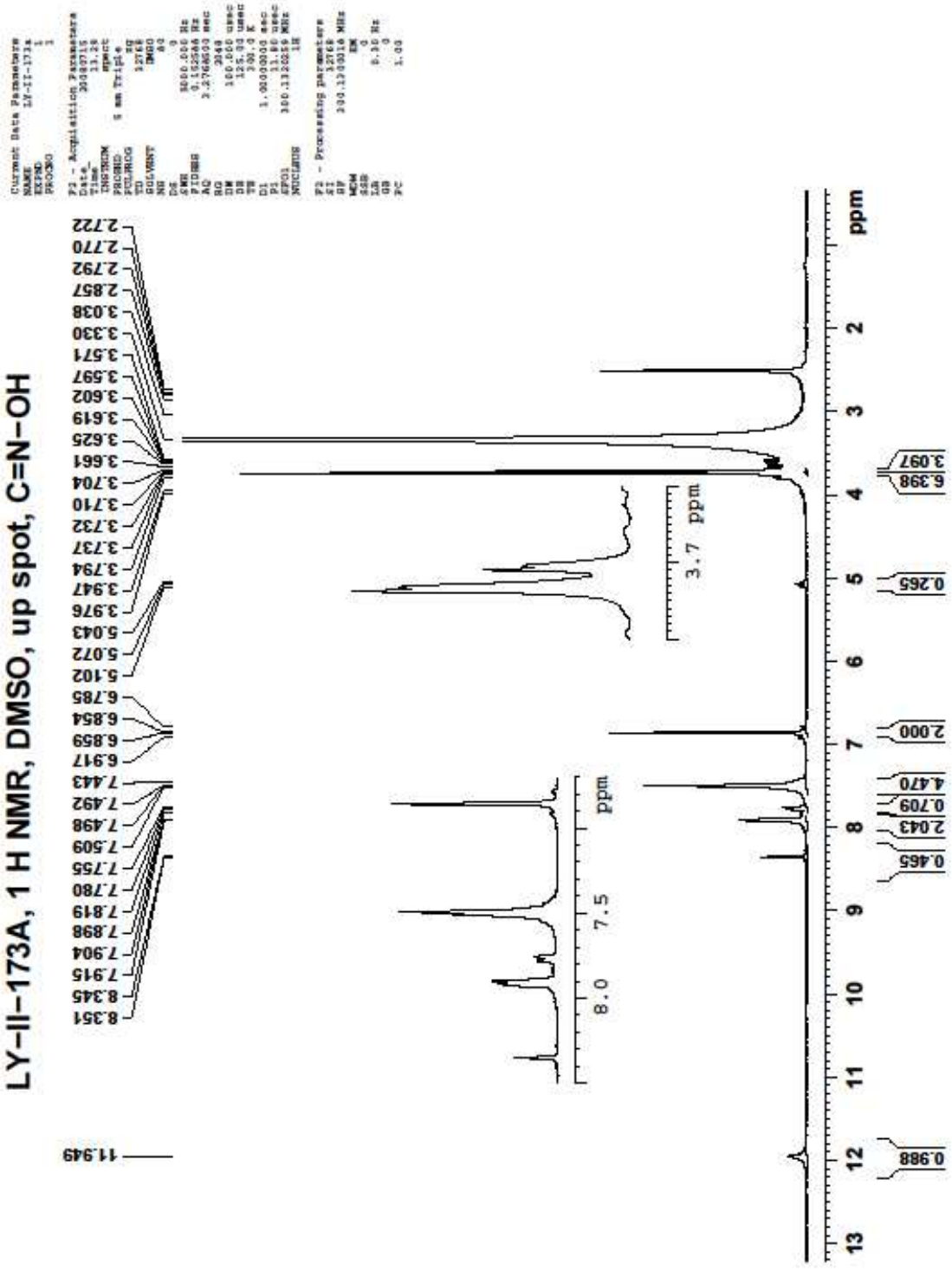
Figure 2. Structure of each isomer with their chemical shifts. Distinct differences in their chemical shifts for atoms between the two isomers are labeled on the structure in red.

When we examined the calculated chemical shifts, several atoms near the region we are interested in showed substantial difference for both protons and carbons, as shown in Table 1. While the calculation could not produce the exact experimental chemical shifts, the trend is very clear, and isomer 1 is assigned as **13a** while isomer 2 is assigned as **13b**.

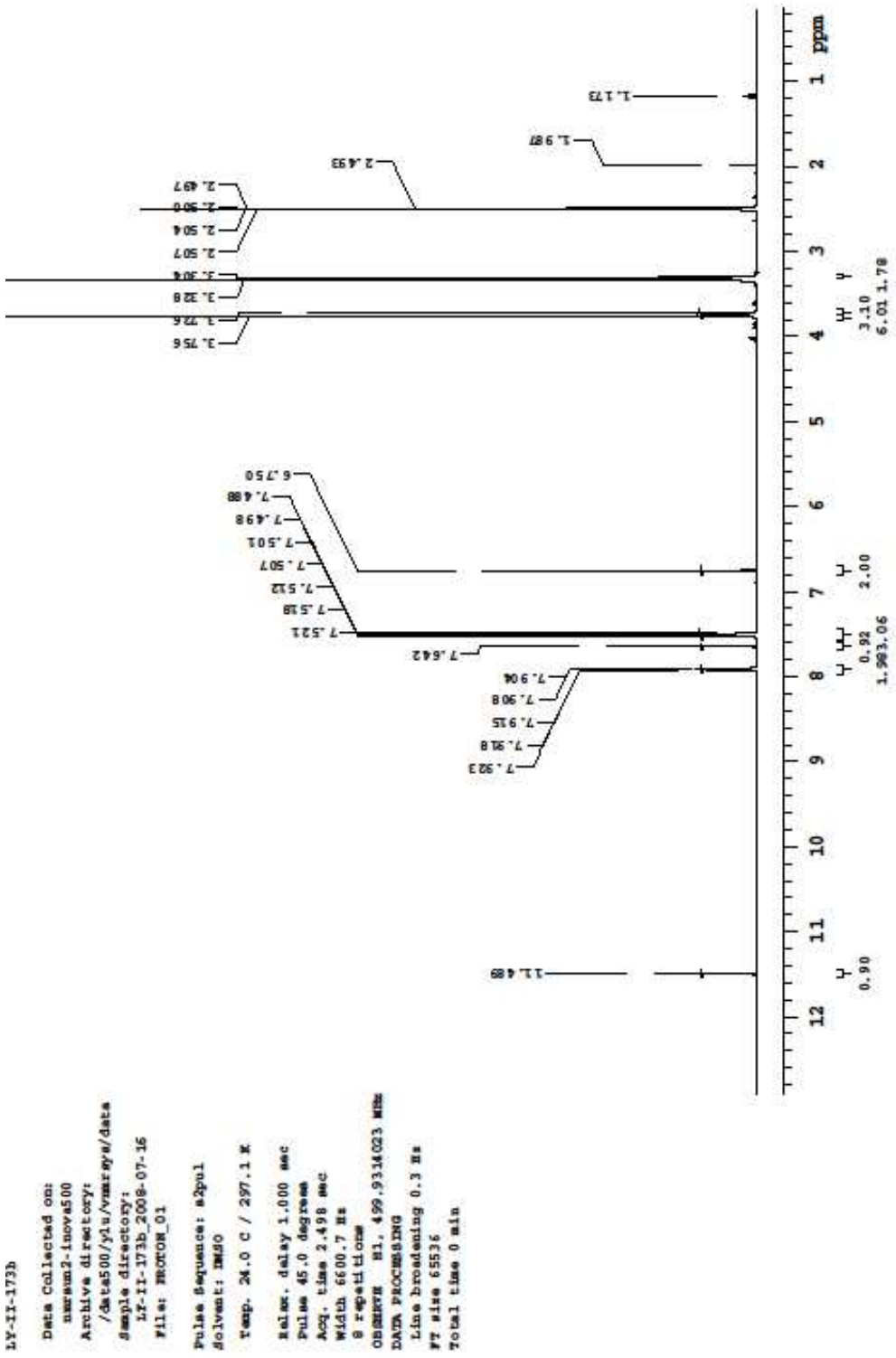
Table 1: Experimental and calculated chemical shifts for key atoms differentiating the two isomers

Atom label	Calculated shifts (ppm)		Experimental shifts (ppm)	
	Isomer 1	Isomer 2	**13a**	**13b**
Ha	8.5	7.5	8.3	7.6
Hb	6.6	6.3	11.9	11.4
Ca	129	120	124.2	119.6
Cb	141	149	145.8	152.8

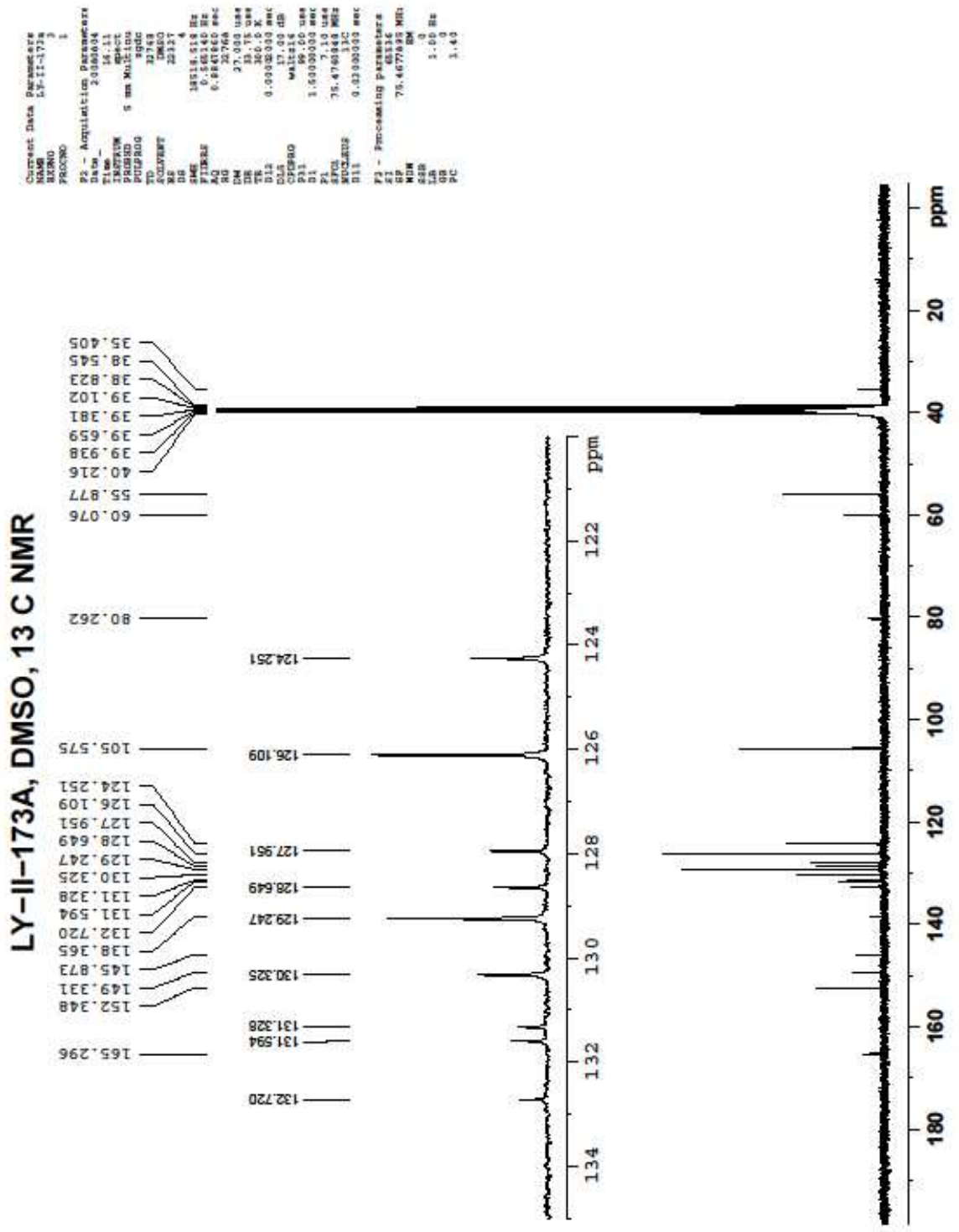
[1]H NMR of isomer **13a** in DMSO-*d6*



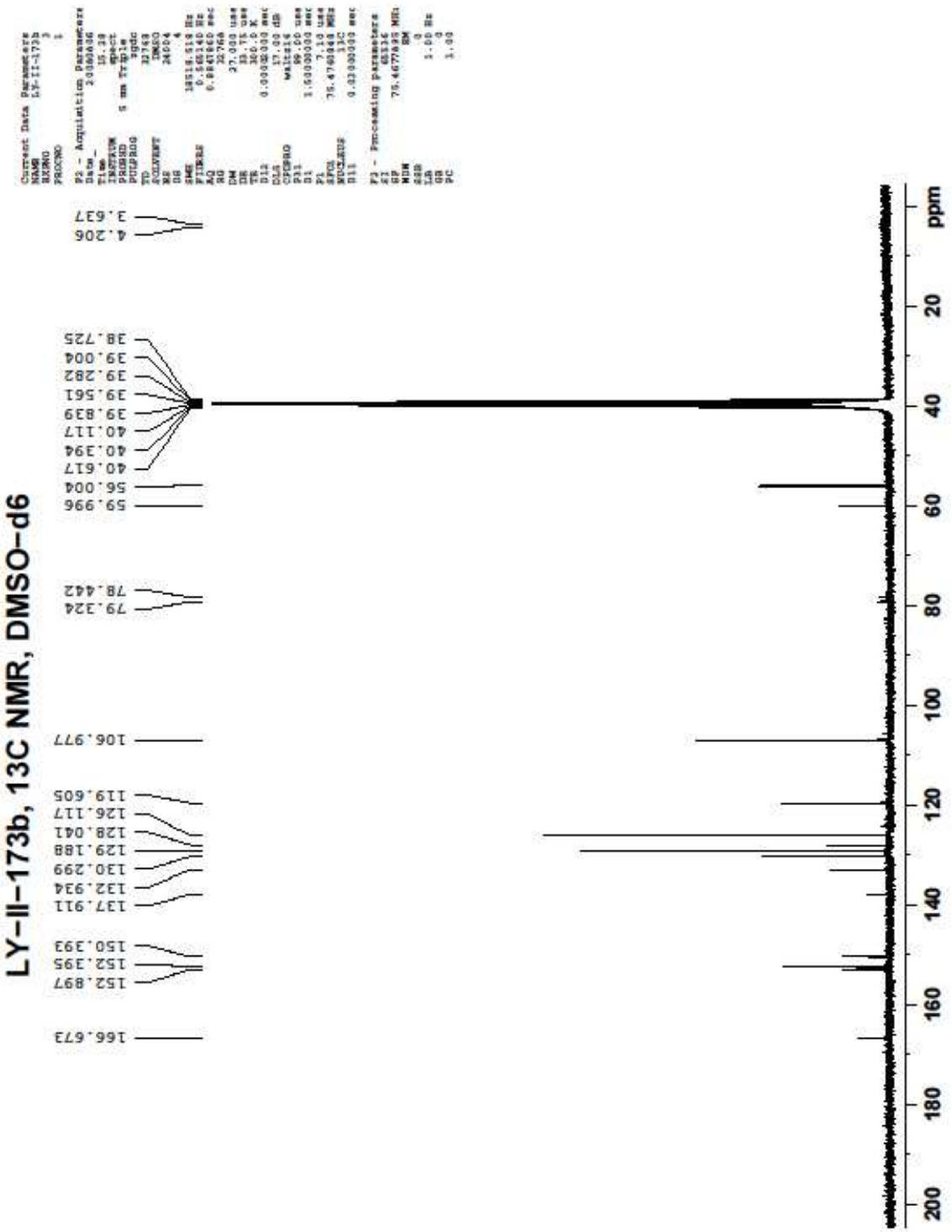
^{1}H NMR of isomer **13b** in DMSO-*d6*



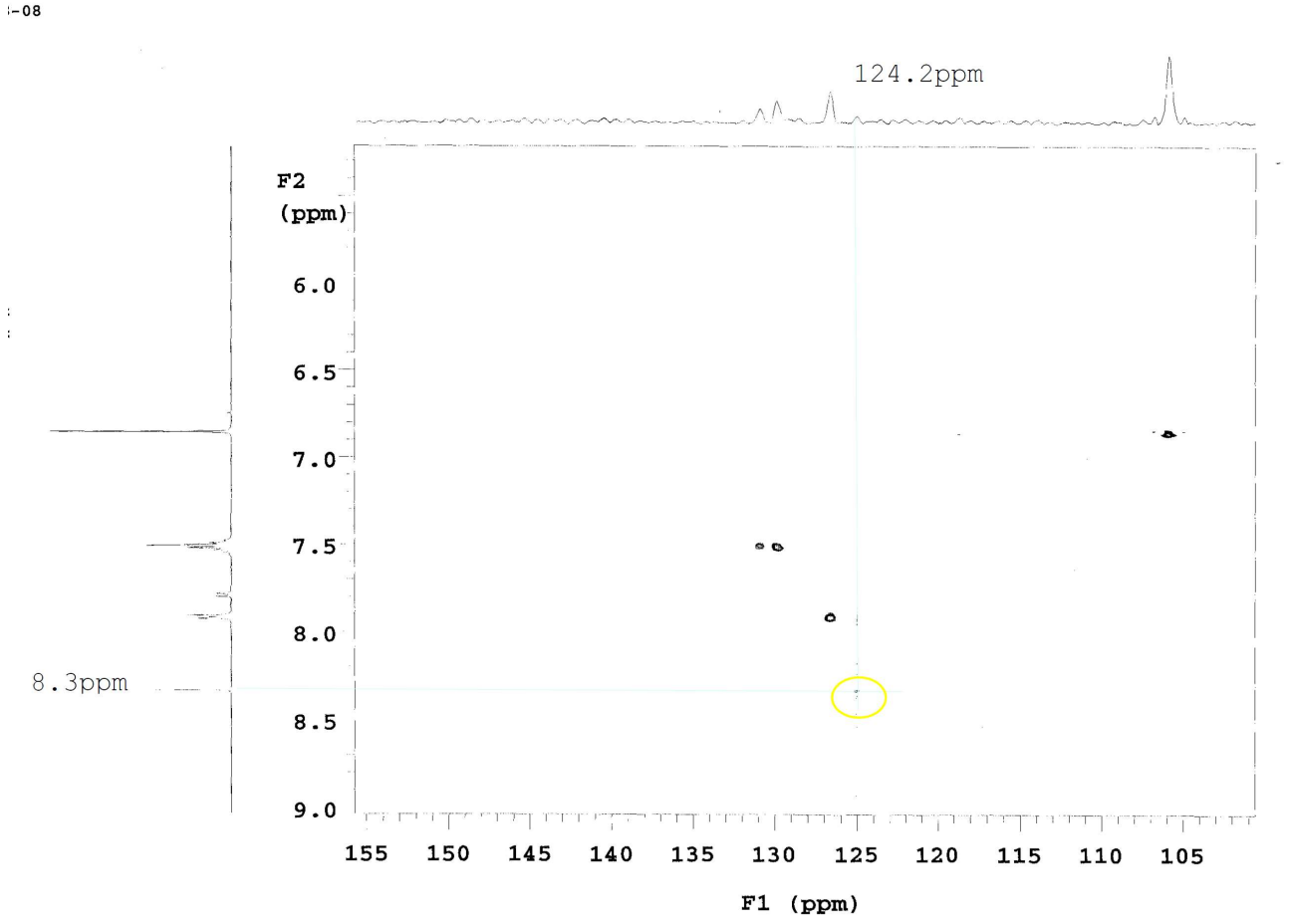
^{13}C NMR of isomer **13a** in DMSO-*d6*



^{13}C NMR of isomer **13b** in DMSO-*d6*

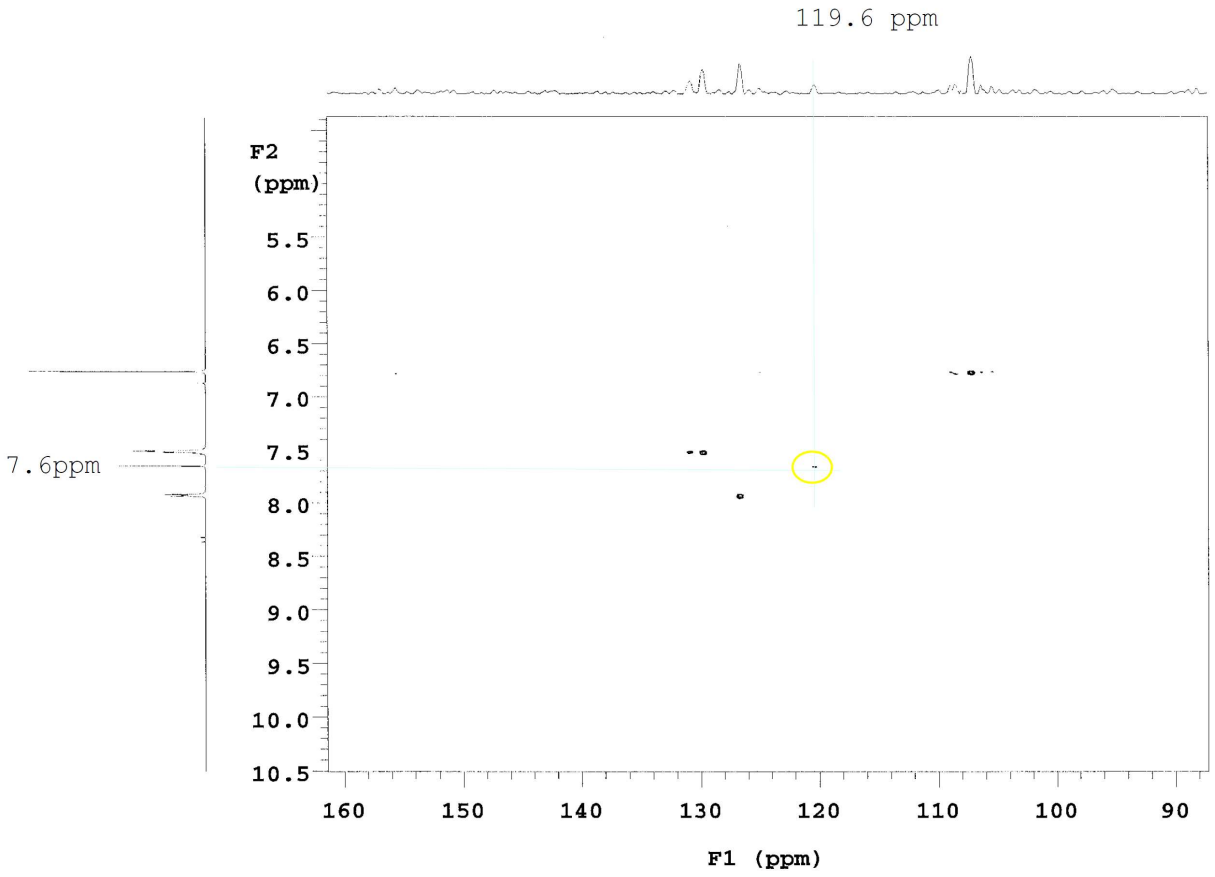


HMQC of isomer **13a** in DMSO-*d6*



HMQC of isomer **13b** in DMSO-*d6*

HMQC of 13b
To find out chemical shift of Ca is 119.6 ppm, which is correlated with Ha at 7.6 ppm in isomer 2



Potential binding mode of PAT at the colchicine site in αβ-tubulin heterodimer

Using molecular modeling, we docked PAT compound **45c** and compared its potential binding mode compared with SMART (**1**) and colchicine. We employed the widely used crystal structure of DAMA-colchicine in tubulin (PDB code: 1SA0) [1] in literature and performed docking studies using Schrodinger Molecular Modeling Suite 2010 (Schrodinger Inc., New York, NY, USA). As an example, the results are shown in following **Figures** for the most active PAT compound **45c**, the corresponding SMART compound **1**, and the native ligand DAMA-colchicine for comparison (**Figure A**). In general, **45c** and **1** bind well to the colchicine binding pocket overlap well with that of the native ligand at the colchicine binding site (**Figure B**). This is consistent with the experimental result and suggests that PAT and SMART may have similar mechanism of action by producing their antiproliferative effects through inhibition of tubulin polymerization.

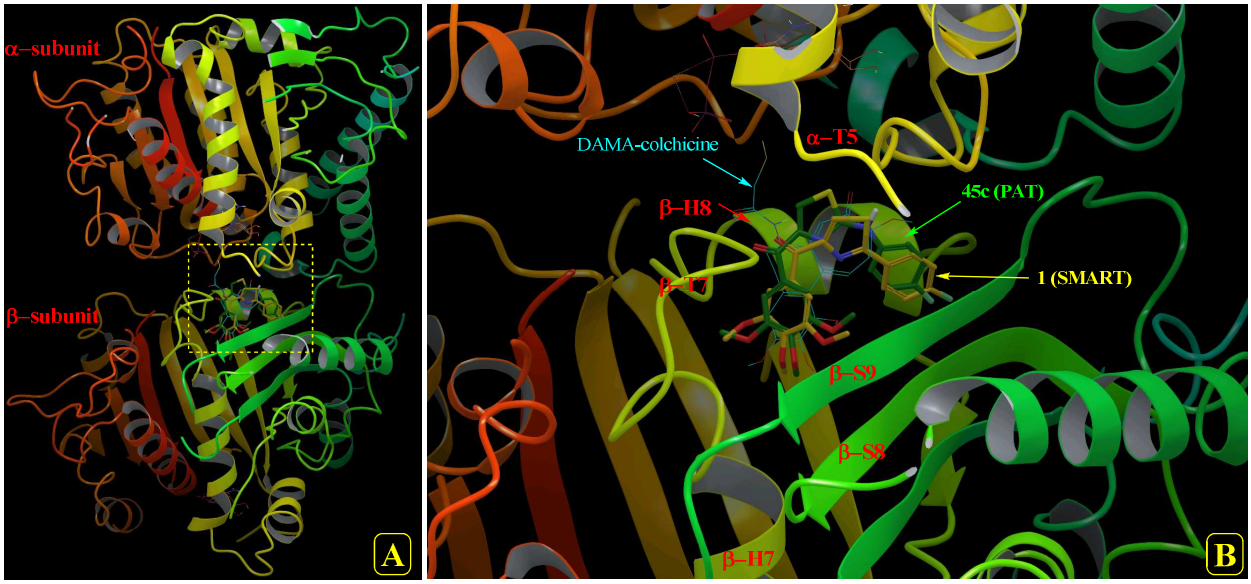


Figure A. Molecular docking model of SMART, PAT and DAMA-Colchicine (the dotted line framed) in α, β-tubulin heterodimer.

Figure B. The overall structures of compound **1** (SMART, Golden stick model), **45c** (PAT, green stick model) and DAMA-colchicine (wire models) overlapped very well in the binding pocket.

[1] Ravelli, R. B.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* **2004,** 428, 198-202.