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SYNTHESIS AND COX-2 INHIBITORY ACTIVITIES OF RUTAECARPINE HOMOLOGUES

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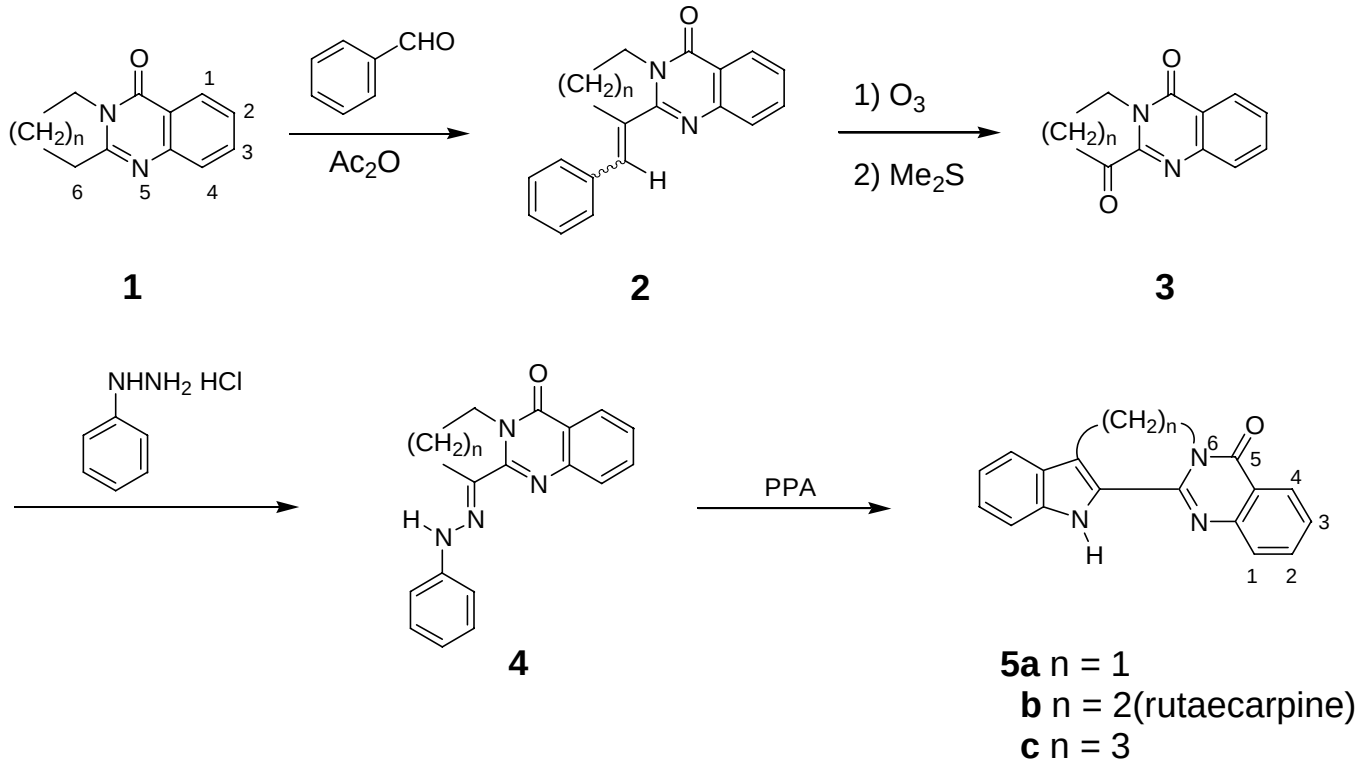
Abstract – Homologous series of rutaecarpine were prepared by structurally modifying the C-ring and were evaluated their inhibitory activities on COX-2. The inhibitory activity on COX-2 increased with the increase of methylene unit while the selectivity on COX-2 over COX-1 decreased to lead a loss in trimethylene bridged system

Rutaecarpine (**5b**)[1] is a major alkaloid constituent of Rutaceous plants which have long been utilized for the treatment of inflammation-related disorders in the traditional oriental medicinal practice.[2] Recent findings of potent and selective inhibitory activity of rutaecarpine on cyclooxygenase-2 (COX-2) provided a rationale for such an anti-inflammatory activity.[3] Continuing interests on rutaecarpine led to not only identification of the additional biological activities such as vasorelaxing,[4] analgesic,[5] antiplatelet,[6] and cytotoxic activities,[7] but also development of the methods aimed towards total synthesis.[8] As a part of our interests in the conformational effect towards the biological activities, we herein described synthesis and biological activities of a homologous series of rutaecarpine in which the dihedral angle between planar indole ring and quinazolinone ring was controlled by the length of methylene unit.

RESULTS AND DISCUSSION

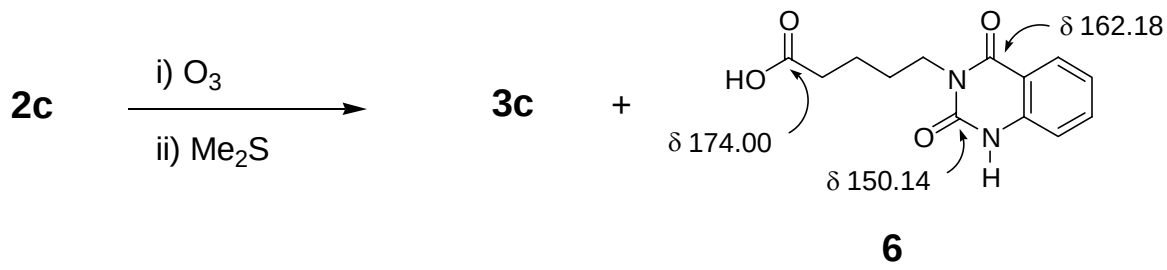
Chemistry: The previously reported method[8f] for the preparation of rutaecarpine (**5b**) by us was applied to the synthesis of its homologues. The prerequisite 2,3-polymethylene-4(3*H*)-quinazolinone (**1a**,**c**),[8c] was thus condensed with benzaldehyde to afford 6-benzylidene derivatives (**2a**,**c**) in 76 and 78% yields, respectively. The *E*- and *Z*-isomers were formed as expected in a ratio of 1.8:1 and 7:1 for **2a** and **2c**, respectively, while *E*-isomer (**2b**) was the only product.[8f] These results reflected the piperidine ring with three sp^2-hybridized atoms in **2b** imposed severe steric congestion in the bay area by phenyl group and

lone pairs of electron of N5 to lead *E*-isomer as an only product. On the other hand, the "flatten out effect" of five-membered ring moiety in **2a** and the "distortion effect" of seven-membered ring in **2c** relieved such congestion somewhat for the *Z*-isomers. Two isomers were readily separated by either recrystallization or column chromatography and assigned by spectroscopic methods. The more CH_2Cl_2 soluble part was assigned to *E*-isomer (**2aa**) based on 7.87% NOE effect between H10 (at δ 3.80) and two *ortho* protons (at δ 7.65) of phenyl group while *Z*-isomer (**2ab**) showed only 3.17%.[9] Ozonolysis of benzylidene compounds (**2a**,**c**) afforded corresponding diketones (**3a**,**c**) in 83 and 68% yields, respectively, after reductive work up. The diketones (**3**) were, then, reacted with phenylhydrazine-HCl to afford the corresponding hydrazones (**4**) in good yields (> 82%). Fischer indolization was applied to these hydrazones to provide the desired rutaecarpine homologues (**5a**)[10] and (**5c**)[11] in 65 and 95% yields, respectively. The increasing nonplanarity of these homologues leads to a monotonic decrease in their melting points: **5a** (>280 ^{o}C), **5b** (259-260 ^{o}C), and **5c** (231 ^{o}C) as similar trend has been previously reported for 3,3'-annelated 2,2'-bipyridines.[12]



It is worthwhile to note that ozonolysis of **2c** has resulted in unexpected result to afford **3c** and **6** in a ratio of 4:1. The structure of **6** was confirmed by spectroscopic methods and elemental analysis. ^{1}H NMR spectrum of **6** showed two D_2O exchangeable proton resonances at δ 12.00 and δ 11.43 for acidic OH and NH, respectively. ^{13}C NMR spectrum showed three C=O resonances at δ 150.14 and δ 162.18 for C2 and

C4, and δ 174.00 for acidic carbonyl, respectively. Four aliphatic carbon resonances at δ 22.17, 27.21, 33.53, and 39.89 were well matched with those (δ 23.08, 28.01, 35.23, and 40.00) of 5-aminopentanoic acid. The mechanism for the formation of **6** remained to be explained.



6

Biological properties: Inhibitory activities of the compounds prepared on cyclooxygenase-1 and 2 (COX-1 and 2) were evaluated as compared to indomethacin and selective COX-2 inhibitor NS-398 by employing previously described method,[3] and summarized in Table 1. The inhibitory activity on COX-1 was significantly increased with the increase of the length of methylene unit while the activity on COX-2 was slightly increased. Selectivity on COX-2, thus, decreased with the increase of length of bridge leading a loss of selectivity in the most distorted **5c**.

Table 1. Inhibitory Activities of Rutaecarpine Homologues on COX-1 and COX-2

Compounds	IC_{50} (μM)		Selectivity (COX-1/COX-2)
	COX-1	COX-2	
5a	98.1	2.5	39
5b	8.7	0.28	31
5c	0.23	0.13	1.8
Indomethacin	0.016	0.009	1.9
NS-398	1.67	< 0.002	> 8,300

In conclusion, a series of rutaecarpine homologues were prepared from 2,3-polymethylene-4(3*H*)-quinazolinones in 4 steps and their inhibitory activities on COX-1 and 2 were evaluated. The inhibitory activity on COX-2 increased with the increase of the length of methylene unit while selectivity decreased leading a loss of selectivity in trimethylene-bridged system.

EXPERIMENTAL

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz for ^{1}H NMR and 62.5 MHz for ^{13}C NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). The starting **1a**, **1c**,[8c] and rutaecarpine (**5b**)[8f] were prepared by employing previously reported method. Chemicals and solvents were commercial reagent grade. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer. The IUPAC nomenclatures of the new compounds prepared were determined using a Chemistry 4-D Draw Pro 3.0 program (ChemInnovation Software, Inc.).

7,8-Dihydro-6-phenylmethylidenepyrrolo[2,1-*b*]quinazolin-10(6*H*)-one (**2a**)

A mixture of 31.23 g (0.17 mol) of **1a** and 53.24 g (0.50 mol) of benzaldehyde in 205 mL of Ac_2O was refluxed for 48 h. Excess benzaldehyde and Ac_2O were removed under reduced pressure. To the residue was added 100 mL of water. Resulting mixture was made basic with 50% aqueous NaOH and poured to CH_2Cl_2 (150 mL). The precipitate formed (21.90 g. 48%) was collected and recrystallized from CH_2Cl_2:Et_2O (1:1) to give **(E)-7,8-dihydro-6-phenylmethylidenepyrrolo[2,1-*b*]quinazolin-10(6*H*)-one** (**2aa**) as pale yellow needles: mp 176-178 ^{o}C (lit.,[13a] mp 161-163 ^{o}C, lit.,[13b] mp 175-176 ^{o}C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.16 (dd, *J* = 8.0, 0.8 Hz, H5), 7,87 (td, *J* = 6.8, 2.5 Hz, 1H, benzylidene H), 7.84 (dt, *J* = 8.4, 1.2 Hz, H8), 7.81 (d, *J* = 8.0 Hz, H4'), 7.65 (d, *J* = 7.6 Hz, 2H, H2'), 7.54 (overlapped t, *J* = 7.2 Hz, 3H, H6 and H3'), 7.34 (t, *J* = 7.6 Hz, H7), 4.21 (t, *J* = 6.8 Hz, 2H), 3.30 (dt, *J* = 6.8, 2.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.07, 155.90, 148.69, 135.22, 134.41, 132.77, 129.80, 129.56, 129.01, 128.95, 126.62, 126.18, 125.83, 120.43, 44.27, 25.14. *Anal*. Calcd for $C_{18}H_{14}N_2O$: C, 78.81; H, 5.14; N, 10.21. Found: C, 78.86; H, 5.24; N, 9.99. The filtrate was washed with water, brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded 13.5 g of a solid material, which was recrystallized from EtOH to give 12.60g (27%) of **(*Z*)-7,8-dihydro-6-phenylmethylidenepyrrolo[2,1-*b*]quinazolin-10(6*H*)-one** (**2ab**) as pale yellow needles: mp 178-180 ^{o}C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, *J* = 8.0 Hz, H5), 7.91 (t, *J* = 6.8 Hz, benzylidene H), 7.80 (d, *J* = 8.0 Hz, H8), 7.77 (t, *J* = 7.8 Hz, *para* H of phenyl), 7.60 (d, *J* = 7.2 Hz, two *ortho* H's of phenyl), 7.50-7.45 (m, 3H, H6 and two *meta* H's of phenyl), 7.41 (t, *J* = 8.0, H7), 4.28 (t, *J* = 7.6 Hz, 2H), 3.30 (dd, = 7.2, 2.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.41, 155.80, 149.75, 135.69, 134.48, 131.68, 131.13, 130.04, 129.23, 129.07, 127.42, 126.61, 126.42, 121.06, 44.29, 25.74. Compound (**2ab**) gave the same analytical data as **2aa**.

7,8,9,10-Tetrahydro-6-phenylmethylideneazepino[2,1-*b*]quinazolin-12(6*H*)-one (**2c**).

The same procedure described for **2a** was employed with 21.4 g (0.1 mol) of **1c** to give 22.7 g of solid which was recrsytallized from EtOH to give 19.9g (68%) of **(*E*)-7,8,9,10-tetrahydro-6-phenylmethylidenepyrrolo[2,1-*b*]quinazolin-12(6*H*)-one** (**2ca**) as white needles: mp 160-161 ^{o}C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.31 (dt, *J* = 8.3, 1.0 Hz, 1H), 7.78-7.74 (m, 3H), 7.53-7.32 (m, 6H), 4.32 (t, *J* = 5.8 Hz, 2H), 2.81 (m, 2H), 1.95-1.88 (m, 4H). *Anal*. Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.27. Found: C, 79.36; H, 5.94; N, 9.35. Concentration of mother liquor afforded 2.80 g (10%) of **(*Z*)-7,8,9,10-tetrahydro-6-phenylmethylidenepyrrolo[2,1-*b*]quinazolin-12(6*H*)-one** (**2cb**) as white needles: mp 139-141 ^{o}C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.23 (dt, *J* = 8.2, 1.2 Hz, 1H), 7.88-7.77 (m, 2H), 7.42-6.50 (m, 7H), 4.36 (t, *J* = 5.8 Hz, 2H), 2.59 (m, 2H), 1.92-1.80 (m, 4H).

7,8-Dihydropyrrolo[2,1-*b*]quinazoline-6,10-dione (**3a**)

A solution of 2.76 g (0.01 mol) of **2ab** in 200 mL of CH_2Cl_2 was cooled in acetone-dry ice bath and ozone was bubbled through the solution until the solution turns blue. Excess ozone was purged and 20 mL of Me_2S was added into the mixture. Evaporation of the solvent afforded 1.76 g of semi-solid, which was chromatographed on silica gel, eluting with CH_2Cl_2. Early eluent gave 1.66 g (83%) of solid material which was recrystallized from CH_2Cl_2:*n*-hexane (1:2) to provide white needles: mp 165 ^{o}C (turned dark without melting) [lit.,[14] mp 165-170 ^{o}C (darkens without melting), 205 ^{o}C (chars without melting)]. Spectroscopic data are identical to those previously reported.[14]

7,8,9,10-Tetrahydroazepino[2,1-*b*]quinazoline-6,12-dione (**3c**)

The same procedure described above for **3a** was employed with 3.02 g (0.01 mol) of **2c** to give a white precipitate when Me_2S was added. This precipitate (0.39 g, 17%) was identified as **5-(1,4-dihydro-2,4-dioxo-2*H*-quinazolin-3-yl)pentanoic acid** (**6**): mp 191-192 ^{o}C. IR (KBr) *v* 3500, 1660, 1604, 1580, 1465, 1340, 1308, 1240, 1195, 1155, 915, 780, 695 cm^{-1}. ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.00 (s, OH, exchangeable with D_2O), 11.43 (s, NH, exchangeable with D_2O), 7.90 (dd, *J* = 7.5, 1.0 Hz, H5), 7.63 (ddd, *J* = 8.0, 7.8, 1.5 Hz, H6), 7.18 (t, *J* = 7.5 Hz, H7), 7.15 (d, *J* = 7.5 Hz, H8), 3.87 (t, *J* = 7.5 Hz, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.63-1.45 (m, 4H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 174.60, 162.18, 150.41, 139.64, 135.18, 128.36, 122.72, 115.34, 113.98, 39.89, 33.53, 27.21, 22.17. *Anal*. Calcd for $C_{13}H_{14}N_2O_4$: C, 59.54; H, 5.38; N, 11.68. Found: C, 59.60; H, 5.42; N, 10.45. Evaporation of the solvent afforded 1.65 g of semi solid material which was recrystallized from ether to give 1.56 g (68%) of white needles: mp 132-133 ^{o}C. IR (KBr) *v* 1670, 1610, 1560, 1450, 1340, 1310, 1250, 1190, 1150 cm^{-1}. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.18 (dd, *J* = 8.0, 1.3 Hz, H5), 8.00 (ddd, *J* = 8.3, 7.0, 1.2 Hz, H6), 7.75 (dd, *J* = 8.3, 0.8 Hz, H8), 7.61 (ddd, *J* = 8,3, 7.2, 1.5 Hz, H7), 4.16 (t, *J* = 5.3 Hz, 2H), 2.77 (dd, *J* = 7.0, 4.5 Hz, 2H), 1.91-1.76 (m, 4H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 198.64, 159.65, 152.80,

146.99, 134.86, 128.26 (two C's), 126.62, 121.85, 41.03, 39.03, 24.67, 19.97. *Anal*. Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.36; H, 5.24; N, 12.31.

7,12-Dihydroindolo[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-5-one (**5a**)[10]

To a solution 2.00 g (0.01 mol) of **3a** in 20 mL of 95% EtOH was slowly added 1.40 g (0.013 mol) of phenylhydrazine-HCl. The yellow precipitate formed was collected as a corresponding hydrazone (**4a**) [2.35 g (81%), mp > 200 ^{o}C (EtOAc)]. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.91 (s, N-H), 8.18 (dd, *J* = 8.0, 0.8 Hz, H1), 7.99-7.89 (m, 2H), 7.58 (td, *J* = 7.3, 1.5 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.34 (t, *J* = 7.5 Hz, 2H), 6.98 (t, *J* = 7.5 Hz, 1H), 4.27 (t, *J* = 6.8 Hz, 2H), 3.08 (t, *J* = 6.8 Hz, 2H). This hydrazone was mixed with 10 g of polyphosphoric acid in a heavy-walled beaker, and heated at 180 ^{o}C for 1.5 h. After cooling, the mixture was made basic with 10% NaOH and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed water, dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a solid material which was recrystallized from EtOAc to provide **5a** as pale yellow needles (1.59 g, 72%): mp > 280 ^{o}C. IR (KBr) *v* 3340 (N-H), 1658 (C=O) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.40 (s, N-H), 8.21 (dd, *J* = 7.5, 0.8 Hz, H4), 7.83 (t, *J* = 7.8 Hz, H2), 7.78 (dd, *J* = 7.5, 0.8 Hz, H1), 7.74 (td, *J* = 8.0, 0.8 Hz, H8), 7.52 (d, *J* = 8.0 Hz, H11), 7.50 (d, *J* = 7.0 Hz, H3), 7.31 (t, *J* = 7.5 Hz, H10), 7.16 (t, *J* = 7.8 Hz, H9), 5.09 (s, 2H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.73, 149.25, 148.98, 142.69, 134.44, 133.79, 126.75, 126.23, 126.10, 125.33, 125.15, 121.66, 120.76, 120.67, 119.86, 113.57, 45.98. *Anal*. Calcd for $C_{17}H_{11}N_3O$: C, 74.71; H, 4.06; N, 15.38. Found: C, 74.66; H, 4.04; N, 15.42.

7,8,9,14-Tetrahydro-5*H*-indolo[2',3':3,4]azepino[2,1-*b*]quinazolin-5-one (**5c**)

The same procedure described above for **5a** was employed with 2.28 g (0.01 mol) of **3c** to yield yellow powder as a corresponding hydrazone (**4c**) [2.77 g (87%), mp 156-157 ^{o}C (EtOAc)]. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.65 (s, N-H), 8.20 (d, *J* = 8.0 Hz, H1), 7.97-7.96 (m, 2H), 7.71-7.63 (m, 1H), 7.51 (overlapped d, *J* = 7.8 Hz, 2H), 7.31 (overlapped t, *J* = 8.0 Hz, 2H), 6.96 (t, *J* = 7.3 Hz, 1H), 4.21 (t, *J* = 5.8 Hz, 2H), 2.76 (br s, 2H), 1.89 (br s, 4H). This hydrazone was treated with 10 g of PPA to yield a solid material which was recrystallized from CH_3OH to give pale yellow needles (2.48 g, 95%): mp 231 ^{o}C (lit.,[11] mp 222-224 ^{o}C). Unreported spectral data are as follows: IR (KBr) *v* 3340 (N-H), 1655 (C=O) cm^{-1}. ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.42 (s, NH), 8.15 (d, *J* = 8.0 Hz, H4), 7.83 (td, *J* = 7.1, 1.1 Hz, H2), 7.72 (d, *J* = 8.0 Hz, H1), 7.57 (d, *J* = 7.8 Hz, H10), 7.52 (d, *J* = 8.5 Hz, H13), 7.48 (td, *J* = 8.0, 1.0 Hz, H3), 7.26 (t, *J* = 8.0 Hz, H12), 7.05 (t, *J* = 7.5 Hz, H11), 4.39 (t, *J* = 6.9 Hz, 2H), 3.11 (t, *J* = 6.9 Hz, 1H), 2.20 (quintet, *J* = 6.9 Hz, 2H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 161.02, 149.30, 147.54, 136.98, 134.73, 128.01, 127.06, 126.97, 126.90, 126.42, 124.95, 120.01, 119.70, 119.48, 119.02, 112.32, 41.91, 25.86, 24.72. *Anal*. Calcd for $C_{19}H_{15}N_3O$: C, 75.73; H, 5.02; N, 13.94. Found: C, 75.67; H, 5.09; N, 14.01.

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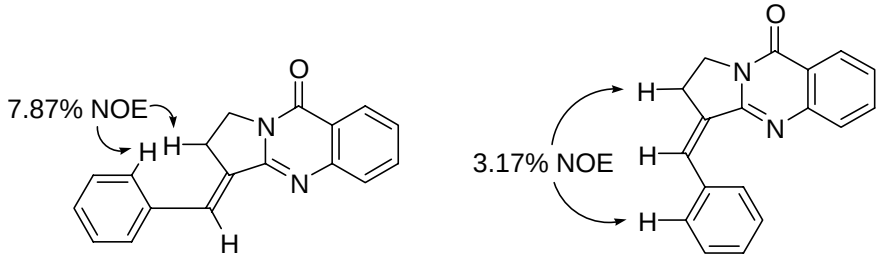

2aa (*E*-isomer) **2ab** (*Z*-isomer)

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