

Tetrazolone as an Acid Bioisostere: Application to Marketed Drugs Containing a Carboxylic Acid

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Supplementary Information

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IMPORTANT SAFETY NOTICE FOR ALL EXPERIMENTS

Azidotrimethylsilane (also known as trimethylsilylazide) is highly toxic and is potentially explosive.¹⁻⁴ Azidotrimethylsilane can also be hydrolyzed to hydrazoic acid (HN₃), a volatile, highly toxic and highly explosive substance.²⁻⁴ Therefore, personnel should be well-versed in the safe-handling and safe disposal of azidotrimethylsilane before attempting to repeat any of the experiments described within this *Supplementary Information*.⁴ It should be noted that all experiments were undertaken behind a blast shield, with personnel wearing appropriate protective clothing for working with azidotrimethylsilane, azide intermediates and tetrazolone products.

It should also be noted that the tetrazolone products arising from these reactions may also be potentially explosive, and possess unknown toxicities. Therefore, appropriate care and safety precautions should also be exercised when performing workups and purifications of the compounds described within this *Supplementary Information*.

1. For a Material Safety Data Sheet (MSDS) on azidotrmethylsilane, see: <http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=155071&brand=ALDRICH&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Faldrich%2F155071%3Flang%3Den>
2. K. J. KerBeek, *Chem. Eng. News*, 1998, **76**, 6.
3. M. Jafarzadeh, *Synlett*, 2007, 2144-2145.
4. For a recent discussion regarding some important considerations when using, or scaling-up azide chemistry, see: F. González-Bobes, N. Kopp, L. Li, J. Deerberg, P. Sharma, S. Leung, M. Davies, J. Bush, J. Hamm, M. Hrytsak, *Org. Proc. Res. Develop.*, 2012, **16**, 2051-2057 and references cited therein.

GENERAL EXPERIMENTAL⁵

All reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions were monitored by thin-layer chromatography or high-performance liquid chromatography. NMR was performed on a 300 MHz NMR spectrometer and all chemical shifts are reported relative to a tetramethylsilane internal standard, or by referencing on the deuterated solvent. Reverse-phase high-performance liquid chromatography was performed on standard equipment and was coupled to diode array and mass spectra detectors – the mass-spectra detector operating under the electrospray ionization (ESI) mode. The column-gradient system was as follows

Column: Phenomenex Gemini 4.6 x 100 mm, C18, 5 μ m, 110Å

Column temperature 30°C

Sample temperature 15°C

Solvent A – 0.05% Formic acid in Water

Solvent B – 0.05% Formic acid in Acetonitrile

Flow rate – 1.5 mL/min

Gradient:

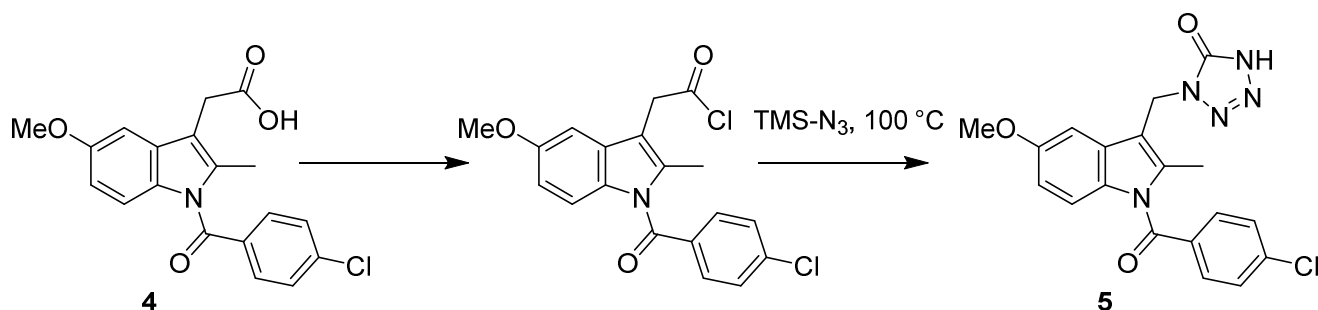
Time	A%	B%
0	95	5
10	0	100 (curve=6)
11.1	0	100
11.2	95	5
12.1	95	5

High resolution mass spectrometry were obtained on a LCT Premier XE mass spectrometer (time-of-flight) operating under the electrospray ionization mode. Mass analysis was performed in extended W-

mode using leucine enkephalin as reference lock mass (556.2771 Da for positive ion & 554.2615 Da for negative ion).

5. The Experimental and data for final compounds is also included in the *Supplemental Information* for an accompanying paper (M. A. J. Duncton, R. Singh, submitted).

PREPARATION OF 1-((1-(4-CHLOROBENZOYL)-5-METHOXY-1*H*-INDOL-3-YL)METHYL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 5



Oxalyl chloride (2.0 M in CH₂Cl₂; 2.3 mL, 4.6 mmol) was added dropwise over 2-3 min to a stirred suspension of 2-(1-(4-chlorobenzoyl)-5-methoxy-1*H*-indol-3-yl)acetic acid, also known as Indomethacin **4** (1.07 g, 3.0 mmol) and DMF (1-2 drops) in CH₂Cl₂ (9 mL) at 0 °C in a vial with pressure-release top. After complete addition, the mixture was allowed to warm to room temperature and stirred at room temperature for *ca.* 60 min. The mixture was concentrated under vacuum to leave the acid chloride, which was used directly in the tetrazolone-forming step below after drying on a high vacuum for 30 min (yield assumed quantitative = 1.13 g).

See ‘Important safety notice for all experiments’ (page S2); Blast shield employed

Note: 4.8 mL (12 equiv.) of azidotrimethylsilane used in order to give sufficient volume for acid chloride to dissolve

A stirred mixture of azidotrimethylsilane (4.8 mL, 18 mmol) and 2-(1-(4-chlorobenzoyl)-5-methoxy-1*H*-indol-3-yl)acetyl chloride (1.13 g, 3.0 mmol) was heated from room temperature to 100 °C (block temperature) in a sealed vial with pressure-release cap. The temperature was lowered to 90 °C and the mixture was stirred at 90 °C overnight (Note: pressure develops during heating). After cooling, the mixture was concentrated under vacuum and then the mixture was dry-loaded on to silica gel by evaporation from EtOAc. The mixture was purified by column chromatography on silica gel (ISCO Combiflash) using hexanes / EtOAc (1:0 to 0:1) as eluent to the product **5** (0.98 g, 82%) as a solid.

^1H NMR (DMSO- d_6 , 300MHz): δ 7.69-7.60 (m, 4H), 7.21 (d, J = 2.4 Hz, 1H), 6.89 (d, J = 9.0 Hz, 1H), 6.73 (dd, J = 9.0, 2.4 Hz, 1H), 5.17 (s, 2H), 3.73 (s, 3H), 2.39 (s, 3H), -1.6 (br. s, 1H)

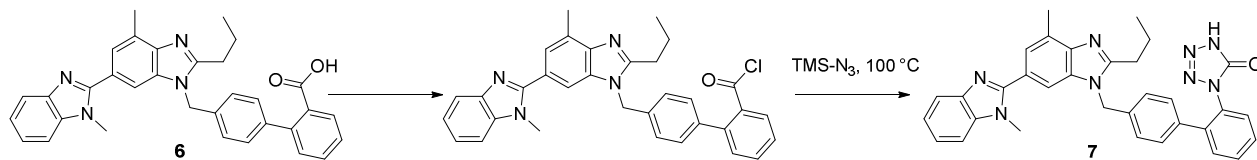
^{13}C NMR (DMSO- d_6 , 75MHz): δ 168.0, 155.6, 151.6, 137.9, 137.2, 133.7, 131.3, 130.3, 129.3, 129.1, 114.7, 113.4, 111.6, 101.6, 55.3, 37.1, 13.0

m/z = 398.41 $[\text{M}+\text{H}]^+$ and 396.53 $[\text{M}-\text{H}]^+$

HRMS (ED): $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{19}\text{H}_{16}\text{ClN}_5\text{O}_3$ m/z 398.1020, found 398.1034

HRMS (ED): $[\text{M}-\text{H}]^+$ calc'd for $\text{C}_{19}\text{H}_{16}\text{ClN}_5\text{O}_3$ m/z 396.0863, found 396.0823

PREPARATION OF 1-(4'-((1,7-DIMETHYL-2'-PROPYL-1*H*,3'*H*-[2,5'-DIBENZO[*D*]IMIDAZOL]-3'-YL)METHYL-[1,1'-BIPHENYL-2-YL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 7



Oxalyl chloride (2.0 M in CH₂Cl₂; 0.75 mL, 1.5 mmol) was added dropwise over 2-3 min to a stirred suspension of 4'-((1,7-dimethyl-2'-propyl-1*H*,3'*H*-[2,5'-dibenzo[*d*]imidazol]-3'-yl)methyl-[1,1'-biphenyl)-2-carboxylic acid, also known as Telmisartan **6** (514 mg, 1.0 mmol) and DMF (3 drops) in CH₂Cl₂ (6 mL) at 0 °C under nitrogen. After complete addition, the mixture was stirred at 0 °C for 10 min, then allowed to warm to room temperature and stirred for 30 min (a yellow then orange solution develops). The mixture was concentrated under vacuum, then fresh CH₂Cl₂ (2 mL) was added to the residue and the mixture concentrated under vacuum again to leave the acid chloride, which was used directly in the tetrazolone-forming step below, after drying on a high vacuum for 15 min (yield assumed quantitative = 533 mg).

See 'Important safety notice for all experiments' (page S2); Blast shield employed

Note: 4.8 mL (36 equiv.) of azidotrimethylsilane used in order to give sufficient volume for the reaction

A stirred mixture of azidotrimethylsilane (4.8 mL, 36 mmol) and 4'-((1,7-dimethyl-2'-propyl-1*H*,3'*H*-[2,5'-dibenzo[*d*]imidazol]-3'-yl)methyl-[1,1'-biphenyl)-2-carbonyl chloride (533 mg, 1.0 mmol) was heated from room temperature to 100 °C (block temperature) in a round bottom flask with reflux condenser under an atmosphere of nitrogen. The mixture was then stirred at 100 °C for 2 hours. After cooling, the mixture was concentrated under vacuum and MeOH was added to the residue. The mixture was dry-loaded on to silica gel and then purified by column chromatography on silica gel (ISCO Combiflash) using CH₂Cl₂ / MeOH (1:0 to 92:8) as eluent to give the pure product **7** (87 mg) and mixed fractions. The mixed fractions were re-purified by column chromatography on silica gel (ISCO Combiflash) using CH₂Cl₂ / MeOH (1:0 to 92:8) as eluent to give the product **7** (97 mg) as a solid. Total

yield of product **7** = 184 mg (33%). Also obtained, was a faster-eluting unidentified by-product (181 mg).

^1H NMR (DMSO- d_6 , 300MHz): δ 7.73 (s, 1H), 7.64-7.47 (m, 7H), 7.29-7.11 (m, 6H), 5.58 (s, 2H), 3.80 (s, 3H), 2.86 (t, J = 7.5 Hz, 2H), 2.61 (s, 3H), 1.75 (sextet, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H), -1.5 (br. s, 1H)

^{13}C NMR (DMSO- d_6 , 75MHz): δ 156.2, 154.0, 151.3, 142.6, 142.4, 139.2, 136.7, 136.7, 136.6, 134.7, 130.9, 130.7, 130.3, 128.8, 128.6, 128.6, 128.3, 126.8, 123.3, 123.2, 122.1, 121.8, 118.6, 110.4, 109.1, 45.9, 31.7, 28.7, 20.7, 16.4, 13.8

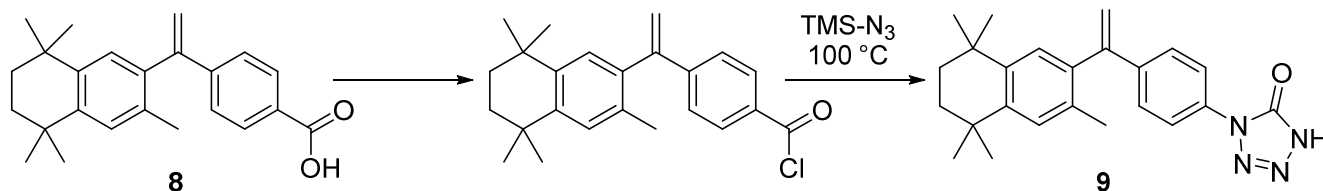
m/z = 555.66 $[\text{M}+\text{H}]^+$ and 553.75 $[\text{M}-\text{H}]^+$

HRMS (EI): $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{33}\text{H}_{30}\text{N}_8\text{O}$ m/z 555.2621, found 555.2585

HRMS (EI): $[\text{M}-\text{H}]^+$ calc'd for $\text{C}_{33}\text{H}_{30}\text{N}_8\text{O}$ m/z 553.2465, found 553.2411

A separate HRMS (EI) obtained: $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{33}\text{H}_{30}\text{N}_8\text{O}$ m/z 555.2621, found 555.2633

PREPARATION OF 1-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)phenyl)-1,4-dihydro-5H-tetrazol-5-one **9**



Oxalyl chloride (2.0 M in CH₂Cl₂; 0.38 mL, 0.75 mmol) was added dropwise over 1 min to a stirred suspension of 4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoic acid, also known as Bexarotene **8** (175 mg, 0.5 mmol) and DMF (1-2 drops) in CH₂Cl₂ (5 mL) at 0 °C under nitrogen. After complete addition, the mixture was allowed to warm to room temperature and for 2 hr. The mixture was concentrated under vacuum to leave the acid chloride, which was used directly in the tetrazolone-forming step below (yield assumed quantitative = 184 mg).

See ‘Important safety notice for all experiments’ (page S2); Blast shield employed

Note: 1.0 mL (15 equiv.) of azidotrimethylsilane used in order to give sufficient volume for the reaction

A stirred mixture of azidotrimethylsilane (1.0 mL, 7.5 mmol) and 4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)benzoyl chloride (184 mg, 0.5 mmol) was heated from room temperature to 100 °C (block temperature) in a 10 mL round-bottom flask under an atmosphere of nitrogen. The mixture was then stirred at 100 °C for 2 hr, by which time LC/MS and TLC analysis indicates completion of the reaction. After cooling, CH₂Cl₂ and MeOH was added to the mixture, which was then dry-loaded on to silica gel and purified by column chromatography on silica gel (ISCO Combiflash) using hexanes / EtOAc (1:0 to 0:1) as eluent to give the product **9** (173 mg, 89%) as a solid.

¹H NMR (DMSO-*d*₆, 300MHz): δ 7.82-7.79 (m, 2H), 7.39-7.36 (m, 2H), 7.13 (s, 1H), 7.06 (s, 1H), 5.84 (s, 1H), 5.18 (s, 1H), 1.91 (s, 3H), 1.63 (br. s, 4H), 1.24 (s, 6H), 1.21 (s, 6H), -1.0 (br. s, 1H)

^{13}C NMR (DMSO- d_6 , 75MHz): δ 150.2, 147.9, 143.7, 141.8, 138.3, 137.9, 133.4, 132.1, 127.9, 127.3, 127.1, 119.5, 115.9, 34.7, 34.6, 33.6, 33.5, 31.7, 31.6, 19.5

$m/z = 389.63$ $[\text{M}+\text{H}]^+$ and 387.68 $[\text{M}-\text{H}]^+$

HRMS (EI): $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{24}\text{H}_{28}\text{N}_4\text{O}$ m/z 389.2341, found 389.2344

HRMS (EI): $[\text{M}-\text{H}]^+$ calc'd for $\text{C}_{24}\text{H}_{28}\text{N}_4\text{O}$ m/z 387.2185, found 387.2211

CLOG P VALUES USING SOFTWARE FROM TWO INDUSTRY-STANDARD PACKAGES

Table S1 compares the clog P values for all compounds using software from ACD Labs and Biobyte Corporation.⁶

Table S1. clog P values using software from two industry-standard software packages

Compound	clog P ^a	clog P ^b
4	4.25 ±0.80	4.2
5	3.85 ±0.79	4.0
6	6.48 ±1.19	7.3
7	6.00 ±1.17	7.1
8	6.90 ±0.53	8.2
9	5.90 ±0.51	7.2

^aclog P values calculated using ACD/Log P version 11.02. ^bclog P values calculated using software from Biobyte within ChemBioDraw Ultra 13.0.

6. For a cautionary comment on clog P values see, D. Lowe, Watch that little letter 'c'.
http://pipeline.corante.com/archives/2012/08/22/watch_that_little_letter_c.php.

BIOLOGICAL ASSAYS AND DATA

The compounds in Table 1 were screened against human variants of COX1, AT1 and RXR α . The evaluation of compounds at hCOX1 and hAT1 were undertaken by Cerep, France (part of the Cerep / Eurofins / Panlabs group of companies). The evaluation of hRXR α was undertaken by Eurofins / Panlabs, Taiwan, Ltd (note: part of the Cerep / Eurofins / Panlabs group of companies). In each experiment, the appropriate reference compound was run concurrently with compounds **4 & 5** (reference compound: Diclofenac), **6 & 7** (reference compound: Saralasin) and **8 & 9** (reference compound: Flurobexarotene). All compounds were run in duplicate, and the experiments were accepted in accordance with Cerep's validation Standard Operating Procedure (Note: Cerep & Eurofins / Panlabs are part of the same group of companies).

The following experimental summaries are taken from the Cerep / Eurofins / Panlabs data reports.

COX1 antagonist screen:

Source: Human recombinant (Sf9 cells)

Stimulant: Arachidonic acid

Stimulant concentration: 4 μ M

Measured component: PGE₂ (prostaglandin E₂)

Incubation: RT / 5min

Detection method: HTRF (Homogeneous Time Resolved Fluorescence)

Reference compound: Diclofenac

Literature reference:⁷

AT1 antagonist screen:

Source: Human recombinant (HEK-293 cells)

Stimulant: Angiotensin-II

Stimulant concentration: 3nM

Measured component: Intracellular $[Ca^{2+}]$

Incubation: RT

Detection method: Flourimetry

Reference compound: Saralasin

Literature reference:⁸

RXR α agonist screen:

Source: Human recombinant (Sf9 cells)

Incubation: RT / 30 min

Detection method: HTRF-coactivator assay (Homogeneous Time Resolved Fluorescence)

Reference compound: Fluorobexarotene

Procedure: Test compound and/or vehicle is preincubated with the 5 nM retinoid X receptor alpha (RXR α)-LBD and coactivator peptide for 30 minutes at 25°C. Determination of the amount of complex formed is read spectrofluorimetrically (excitation:340 nm, emission:520/495 nm). Test compound-induced increase fluorescence by 50 percent or more ($\geq 50\%$) relative to the 10 μ M fluorobexarotene response indicates possible RXR α receptor agonist activity.

Literature reference:⁹

The results are expressed as a percent of control agonist response

$$\frac{\text{measured response}}{\text{control response}} * 100$$

and as a percent inhibition of control agonist response

$$100 - \left(\frac{\text{measured response}}{\text{control response}} * 100 \right)$$

obtained in the presence of the test compounds.

The EC₅₀ values (concentration producing a half-maximal response) and IC₅₀ values (concentration causing a half-maximal inhibition of the control agonist response) were determined by non-linear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting

$$Y = D + \left[\frac{A - D}{1 + (C/C_{50})^{nH}} \right]$$

Where, Y = response, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, and C₅₀ = EC₅₀ or IC₅₀, and nH = slope factor.

This analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot[®] 4.0 for Windows[®] (© 1997 by SPSS Inc.).

For the antagonists, the apparent dissociation constants (K_B) were calculated using the modified Cheng Prusoff equation,

$$K_B = \frac{IC_{50}}{1 + (A/EC_{50A})}$$

Where, A = concentration of reference agonist in the assay, and EC_{50A} = EC_{50} value of the reference agonist.

7. K. Glaser, M. L. Sung, K. O'Neil, M. Belfast, D. Hartman, R. Carlson, A. Kreft, D. Kubrak, C. L. Hsiao, B. Weichman, *Eur. J. Pharmacol.*, 1995, **281**, 107-111.

8. M. M. Martin, B. M. Willardson, G F. Burton, C. R. White, J. N. McLaughlin, S. M. Bray, J. W. Ogilvie, T. S. Elton, *Mol. Endocrinol.*, 2001, **15**, 281-293.

9. C. E. Wagner, P. W. Jurutka, P. A. Marshall, T. L. Groy, A. van der Vaart, J. W. Ziller, J. K. Furmick, M. E. Graeber, E. Matro, B. V. Miguel, I. T. Tran, J. Kwon, J. N. Tedeschi, S. Moosavi, A. Danishyar, J. S. Philp, R. O. Khamees, J. N. Jackson, D. K. Grupe, S. L. Badshah, J. W. Hart, *J. Med. Chem.*, 2009, **52**, 5950-5966.

INHIBITION OF COX1 WITH INDOMETHACIN **4** AND COMPOUND **5**

Indomethacin **4** and compound **5** were screened against hCOX1 at a concentration of 1 μ M in duplicate.

The results are shown below.

Antagonist response for compound **4** at 1 μ M = 88.1% (individual response 92.4% & 83.7%)

Antagonist response for compound **5** at 1 μ M = 36.6% (individual response 28.4% & 44.8%)

Reference compound used in experiment: Diclofenac

DOSE-RESPONSE FOR COMPOUND 7 IN AT₁ ANTAGONIST SCREEN

Data for compound 7: IC₅₀ = 1.7nM; K_b = 0.14nM

Individual data points for compound 7 (average of duplicate):

Agonist response to Angiotensin II in presence of compound 7 at 3μM = 3.6% (individual response 3.4% & 3.7%)

Agonist response to Angiotensin II in presence of compound 7 at 0.3μM = 2.5% (individual response 2.3% & 2.6%)

Agonist response to Angiotensin II in presence of compound 7 at 0.1μM = 0.9% (individual response 0.7% & 1.1%)

Agonist response to Angiotensin II in presence of compound 7 at 30nM = 0.8% (individual response 1.1% & 0.5%)

Agonist response to Angiotensin II in presence of compound 7 at 10nM = 2.7% (individual response 0.7% & 4.8%)

Agonist response to Angiotensin II in presence of compound 7 at 3nM = 27.7% (individual response 27.0% & 28.4%)

Agonist response to Angiotensin II in presence of compound 7 at 1nM = 72.0% (individual response 77.9% & 66.2%)

Agonist response to Angiotensin II in presence of compound 7 at 0.1nM = 90.6% (individual response 91.5% & 89.8%)

Reference compound results (run alongside Telmisartan 6 and compound 7): Saralasin IC₅₀ = 1.2nM; K_b = 0.098nM

DOSE-RESPONSE FOR TELMISARTAN **6** IN AT₁ ANTAGONIST SCREEN

Data for compound **6** (Telmisartan): $IC_{50} = 5.4nM$; $K_b = 0.44nM$

Individual data points for compound **6** (Telmisartan) (average of duplicate):

Agonist response to Angiotensin II in presence of compound **7** at $1\mu M = 3.0\%$ (individual response 3.3% & 2.8%)

Agonist response to Angiotensin II in presence of compound **7** at $0.1\mu M = 3.5\%$ (individual response 4.1% & 2.9%)

Agonist response to Angiotensin II in presence of compound **7** at $30nM = 8.2\%$ (individual response 9.5% & 6.9%)

Agonist response to Angiotensin II in presence of compound **7** at $10nM = 27.7\%$ (individual response 27.9% & 27.5%)

Agonist response to Angiotensin II in presence of compound **7** at $3nM = 69.9\%$ (individual response 75.5% & 64.3%)

Agonist response to Angiotensin II in presence of compound **7** at $1nM = 82.1\%$ (individual response 78.5% & 85.8%)

Agonist response to Angiotensin II in presence of compound **7** at $0.3nM = 92.6\%$ (individual response 95.5% & 89.7%)

Agonist response to Angiotensin II in presence of compound **7** at $0.03nM = 98.1\%$ (individual response 98.2% & 98.1%)

Reference compound results (run alongside Telmisartan **6** and compound **7**): Saralasin $IC_{50} = 1.2nM$; $K_b = 0.098nM$

It should be noted that replacement of the acid in Telmisartan, with a tetrazole ring, results in a compound with an $IC_{50} = 13$ nM (compared to IC_{50} of 3 nM measured for Telmisartan).¹⁰

10. U. J. Ries, G. Mihm, B. Narr, K. M. Hasselbach, H. Wittneben, M. Entzeroth, J. C. A. van Meel, W. Wienen, N. H. Huel, *J. Med. Chem.*, 1993, **36**, 4040-4051.

DOSE-RESPONSE FOR COMPOUND 9 IN RXR α CO-ACTIVATOR ASSAY

Individual results for compound 9 (average of duplicate - individual responses not forwarded):

Agonist response for compound 9 at 3 μ M = 84%

Agonist response for compound 9 at 1 μ M = 83%

Agonist response for compound 9 at 0.3 μ M = 76%

Agonist response for compound 9 at 0.1 μ M = 56%

Agonist response for compound 9 at 0.03 μ M = 32%

Agonist response for compound 9 at 10nM = 32%

Reference compound results (run alongside Bexarotene 8 and compound 9): Fluorobexarotene EC₅₀ = 8.6 nM.

Data for Bexarotene 8 in the above assay when run alongside compound 9 and fluorobexarotene control:
EC₅₀ <10 nM

Individual results for Bexarotene 8 (average of duplicate - individual responses not forwarded):

Agonist response for Bexarotene 8 at 3 μ M = 84%

Agonist response for Bexarotene 8 at 1 μ M = 87%

Agonist response for Bexarotene 8 at 0.3 μ M = 83%

Agonist response for Bexarotene 8 at 0.1 μ M = 75%

Agonist response for Bexarotene 8 at 0.03 μ M = 67%

Agonist response for Bexarotene **8** at 10nM = 63%

Reference compound results (run alongside Bexarotene **8** and compound **9**): Fluorobexarotene EC_{50} = 8.6 nM.

Bexarotene **8** and compound **9** showed a similar agonist response at both 3 μ M and 1 μ M. Therefore, the compounds were deemed to show similar relative efficacy at hRXR α .

MICROSOMAL STABILITY STUDIES

Note: The experiment was conducted in duplicate to obtain n=2 for each timepoint.

Microsomal stability studies utilized BD Genquest liver microsomes and were conducted according to the following protocol:

Liver microsomes (20 mg/mL) were thawed, then diluted in sodium phosphate buffer (pH 7.4; 100mM) to a final concentration of 1mg/mL of microsomes. 1µl of 1mM DMSO stock solution of test article (TA) was added to 1mL of microsomal solution (to give a final test article concentration of 1 µM). From this parent solution, an 195µl aliquot was dispensed to a 250µl 96-well plate. The microsome/TA solution was allowed to pre-incubate for 3-5min. Following this pre-incubation period, the reaction was initiated by addition of 5µl of 40mM NADPH (in 100mM phosphate buffer, final concentration 1mM of NADPH) to each well. Immediately after addition of NADPH, a 25µl aliquot was taken and quenched in 50µl ice / acetonitrile containing an internal standard (for Telmisartan **6** and R000 **7**, an in-house internal standard was used; for all other compounds utilizing negative mode, diclofenac was used as the internal standard). Additional 25µl aliquots were taken and quenched from the microsome/TA/NADPH reaction wells at 5, 15, 30, and 45 min timepoints. After quenching, samples were then diluted with 75µl HPLC grade water to give a final acetonitrile content of approximately 33%. Samples were then quantitated via LC/MS. Stability was determined plotting TA disappearance over time on a semilogarithmic plot and calculating the half-life ($t_{1/2}$) from the following equation:

$$t_{1/2} = -\ln 2 / (\text{Slope})$$

Compounds with $t_{1/2}$ exceeding 45 min were reported as Half-life ≥ 45 min.

Also present in each set of experiments were propranolol and/or midazolam, used as positive controls

PLASMA PROTEIN BINDING STUDIES

Note: The experiment was conducted in triplicate to obtain n=3 for each timepoint.

Percentage of compound bound to plasma protein was determined using Life Technologies (of Thermo Scientific, Research Triangle Park, NC) Single-Use RED (rapid equilibration dialysis) device. In brief, the assay was prepared by adding 500µl of Phosphate-Buffered Saline (PBS) solution¹¹ (Mediatech, Herndon, VA) to the buffer side of plate, and 300µl of plasma containing 2µM of the test article (TA) to the plasma side of the plate. Samples were then vortexed at approximately 800rpms for four hours. Following this time period, 50µl plasma and 50 µL buffer were removed from their respective wells and added to a 1.2mL 96-well plate. To the plasma portion was added 50µl of blank PBS buffer, and to the plasma portion was added 50µl of blank plasma to normalize the samples. Next, 300µl of acetonitrile, containing internal standard (for Telmisartan **6** and R000 **7**, an in-house internal standard was used; for all other compounds utilizing negative mode, diclofenac was used as the internal standard). The samples were then vortexed and centrifuged (3000 rpm). Supernatant from each well was then transferred to a 96-deep-well plate and analyzed via LC/MS.

Also present in each set of experiments was wafarin as a positive control

11. This solution is sold under the brand-name of PBS 1x

RAT PHARMACOKINETIC STUDIES

Statement regarding in-life testing: All animals were housed and handled in accordance with the *Guide for the Care and Use of Laboratory Animals*.¹² All procedures for this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Rigel Pharmaceuticals, Inc.

Note: The experiment was conducted in triplicate (3 animals) to obtain n=3 for each timepoint.

Standard stock solutions of Telmisartan **6**, or R000 **7**, in DMSO were prepared in the following manner:

1mg of test article (Telmisartan **6**, or R000 **7**) was dissolved in 1 mL of DMSO to provide a solution of 1×10^6 ng/mL

10 μ L of the 1×10^6 ng/mL DMSO stock solution was diluted with 990 μ L of DMSO to provide a stock solution of 10,000 ng/mL (S1)

The solution of DMSO containing 10,000ng/mL of test article (S1) was used to provide further stock solutions in DMSO

200 μ L S1 + 200 μ L DMSO: 5,000ng/mL (S2)

200 μ L S1 + 800 μ L DMSO: 2,000ng/mL (S3)

100 μ L S1 + 900 μ L DMSO: 1,000ng/mL (S4)

50 μ L S1 + 950 μ L DMSO: 500ng/mL (S5)

20 μ L S1 + 980 μ L DMSO: 200ng/mL (S6)

10 μ L S1 + 990 μ L DMSO: 100ng/mL (S7)

5 μ L S1 + 995 μ L DMSO: 50ng/mL (S8)

Solution S7 was used to provide further stock solutions in DMSO

200 μ L S7 + 800 μ L DMSO: 20ng/mL (S9)

100 μ L S7 + 900 μ L DMSO: 10ng/mL (S10)

Standard controls were then prepared from the DMSO stock solutions above (S1-S10) by adding 10 μ L of each stock solution of DMSO (containing either Telmisartan **6**, or R000 **7**) to 50 μ L blank rat plasma¹³ and then adding 200 μ L of CH₃CN (containing 500ng/mL of an in-house internal standard).

R000 **7** formulations were prepared in the following manner: 5.67 mg of R000 **7** was placed in a 15ml scintillation vial and dissolved with 1ml 0.5N NaOH (fisher scientific); 6ml of saline solution was then added to the formulation and the pH was adjusted to 9.30 using stock solutions of HCl and NaOH (1.1ml added). The resultant formulation had a final volume of 8.1ml and concentration of 0.7mg/mL. SD rats were then dosed with 1ml/kg R000 **7** for a 0.7mg/kg dose IV and 5ml/kg for a 3.50mg/kg PO dose.

Telmisartan **6** was prepared in the same manner, with 6.56 mg added to 15ml scintillation vial, and dissolved in 1ml 0.5N NaOH. The formulation was then brought to 7ml by the addition of 6ml of saline solution and the pH was adjusted to 9.30 through the addition of HCl and NaOH (a total of 1.3ml) for a final volume of 8.3ml and concentration of 0.79mg/mL. Telmisartan **6** was dosed in a similar fashion, with 1mg/mL given to SD rats IV for a 0.79mg/kg dose, and 5ml/kg given PO for a 3.95mg/kg dose.

Rat plasma samples for PK determination were taken at 0.25h, 1h, 1.5h, 2h, 4h, 6h, 8h, 10h, 24h (approximately 200 μ L) from Sprague Dawley (SD) rats and stored at -80°C until ready for analysis.

Samples were thawed in water at room temperature and then lightly vortexed for homogeneity.

50 μ L of plasma from the lightly vortexed rat PK samples was then added to 200 μ L of CH₃CN (containing 500ng/mL of an in-house internal standard).

After addition of the CH₃CN, the standard controls and rat PK samples were vortexed then centrifuged for 15min at 3000 rpms. Supernant was then transferred to a 96-well plate for LC/MS analysis on an API 4000Qtrap mass spectrometer. Samples were analyzed according to the LC/MS method below.

The standard controls allowed construction of a standard curve, which was then used for determination of analyte concentrations in the rat plasma PK samples.

Analyte	Mass parent [M+H] ⁺ Q1	Mass of daughter [M+H] ⁺ Q3	Dwell time (msec)	Declustering Potential DP (Volts)	Collision Energy CE (Volts)	Exit Potential CXP (Volts)	Ionization Energy (Volts)	Temp (°C)
Telmisartan 6	515.155	497.2	100	156	43	6	5500	550
R000 7	555.250	484.2	100	71	33	6	5500	550
Internal standard	397.101	341.1	150	106	53	18	5500	550

Column: Essensil AF-C18 3µM 2.1x50mm AF-C18 column

Mobile phase:

A: Water w/0.05% formic acid

B: Acetonitrile w/0.05% formic acid

Flow rate: 0.6mL/min

Time min	%A	%B
0	95%	5%
0.5	95%	5%
3.5	5%	95%
4.20	5%	95%
4.30	95%	5%
5.0	95%	5%

12. Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. 8th Ed; National Academies Press: Washington (DC), 2011.

13. Blank Rat Plasma: bioreclemation, pooled, non-filtered, sodium heparin

AVERAGE IV CONCENTRATIONS FOR 0.7MG/KG IV DOSE OF R000 7, IN SPRAGUE-DAWLEY RATS

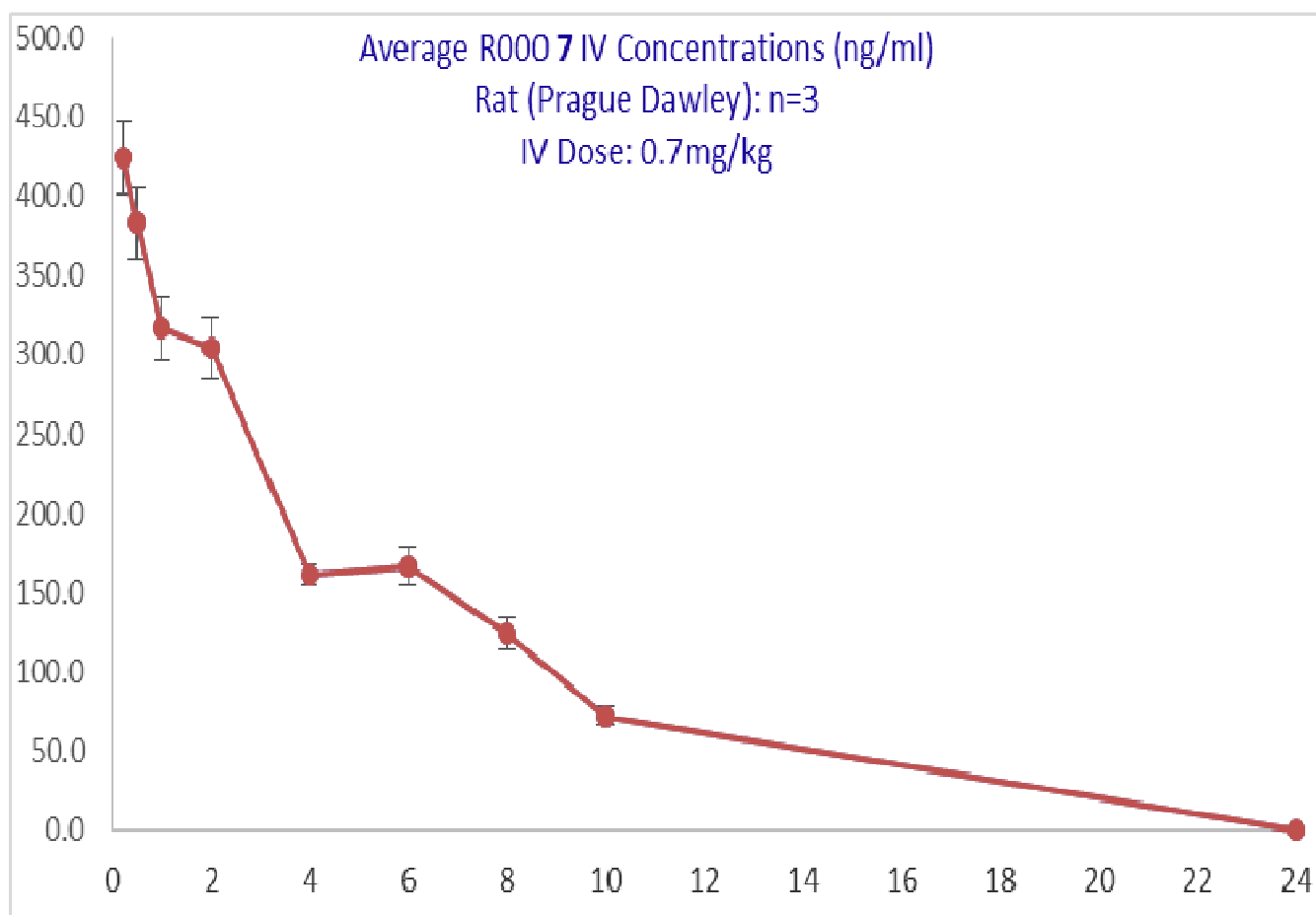


Table S1. Rat pharmacokinetics for tetrazolone compound 7, when dosed by iv route

Compound	7
i.v.	
dose (mg/kg)	0.70
Cl (ml/min/kg)	4.5 ±0.6
AUC _{0-24h} (ng/ml/h)	2490 ±249
AUC _{inf} (ng/ml/h)	2610 ±326
V _{ss} (L/kg)	1.7 ±0.2
Half-life (h)	5.4 ±1.6

^aData is the mean value ±standard deviation

AVERAGE IV CONCENTRATIONS FOR 3.5MG/KG ORAL DOSE OF R000 7, IN SPRAGUE-DAWLEY RATS

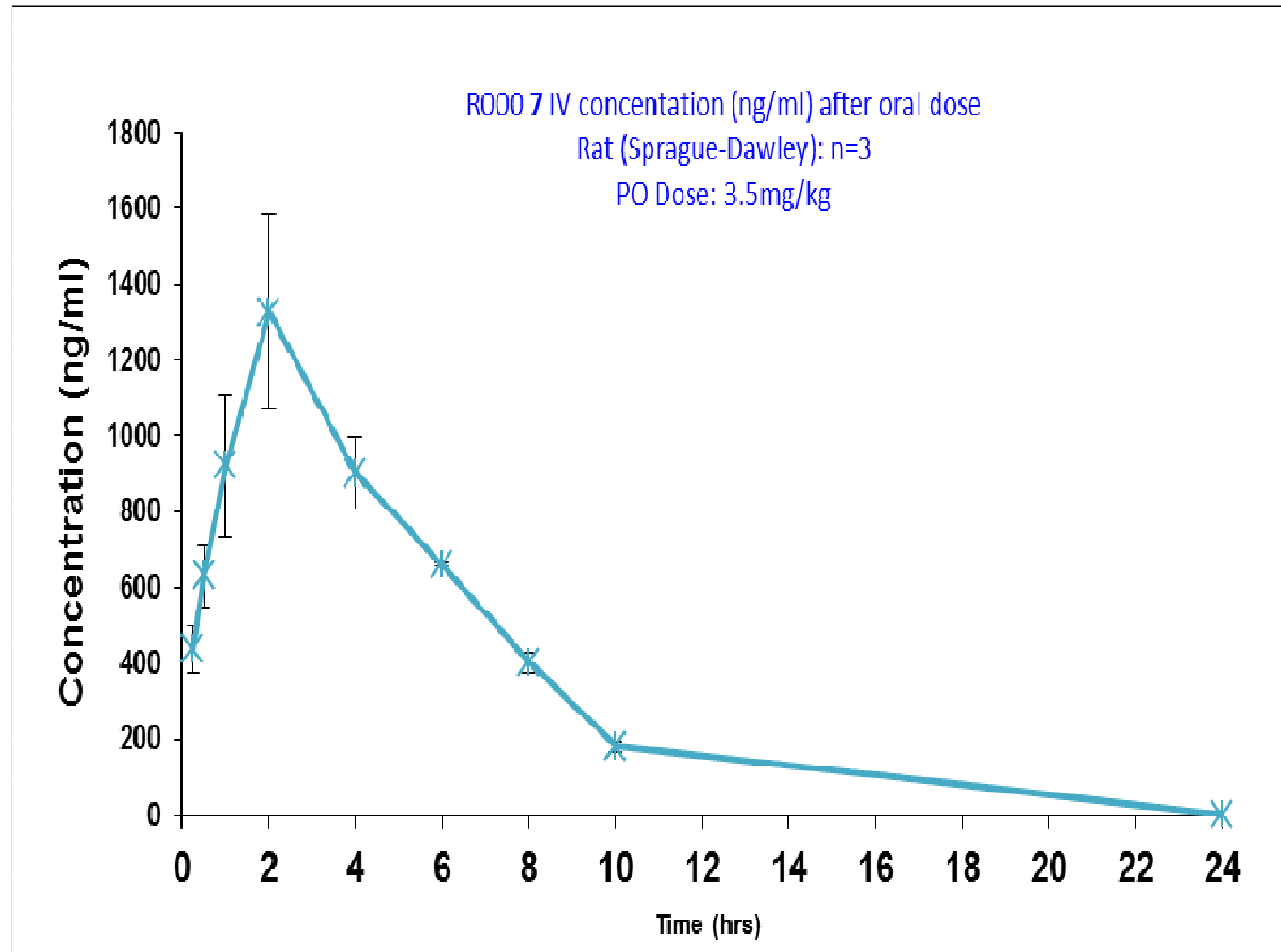


Table S2. Rat pharmacokinetics for tetrazolone compound 7, when dosed by oral route

Compound	7
p.o.	
dose (mg/kg)	3.5
C _{max} (ng/ml)	1330 ±515
AUC _{0-24h} (ng/ml/h)	8420 ±1660
AUC _{inf} (ng/ml/h)	8440 ±1660
F (%)	65

^aData is the mean value ±standard deviation

AVERAGE IV CONCENTRATIONS FOR 0.79MG/KG IV DOSE OF TELMISARTAN 6, IN SPRAGUE-DAWLEY RATS

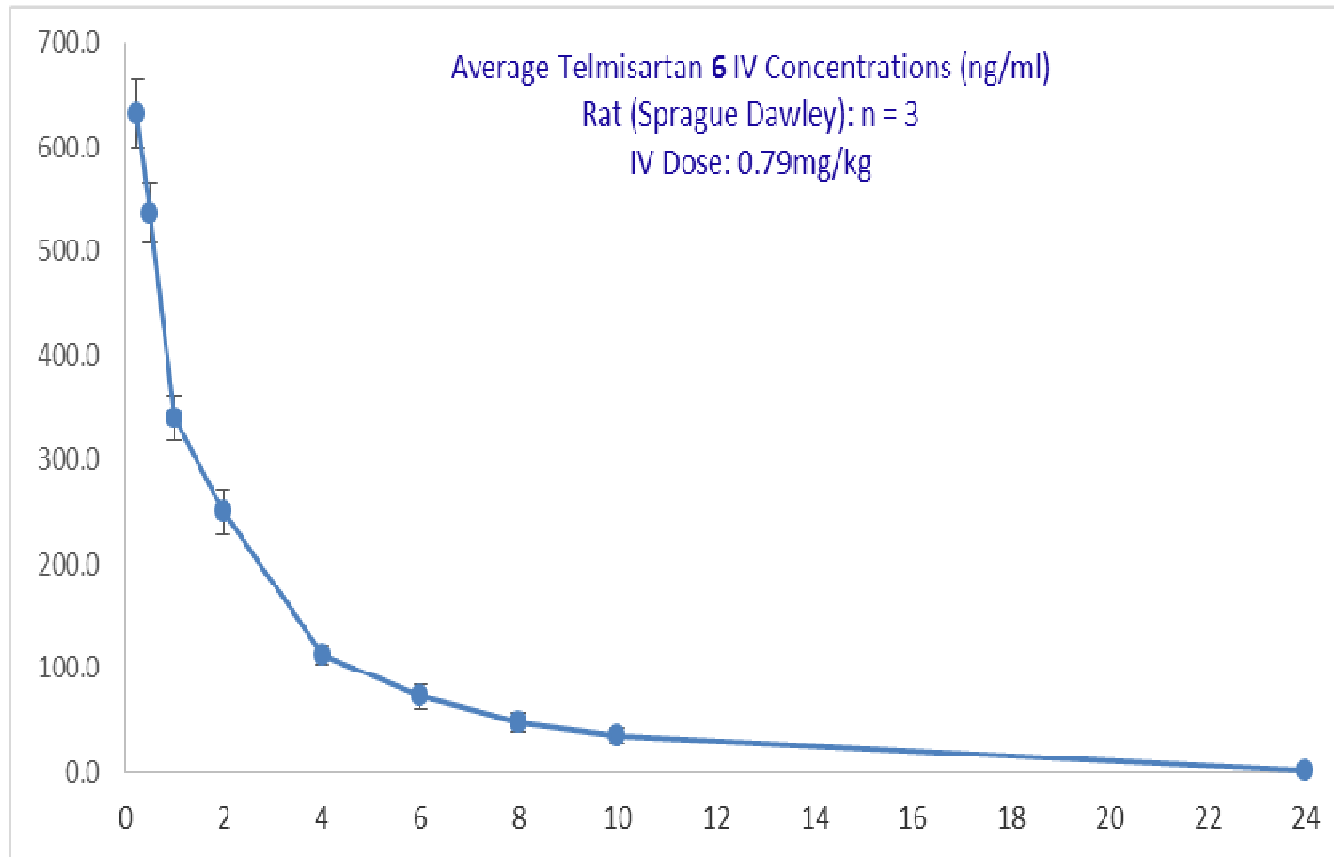


Table S3. Rat pharmacokinetics for Telmisartan 6, when dosed by iv route

Compound	6
i.v.	
dose (mg/kg)	0.79
Cl (ml/min/kg)	7.2 ±1.1
AUC _{0-24h} (ng/ml/h)	1830 ±245
AUC _{inf} (ng/ml/h)	1850 ±258
V _{ss} (L/kg)	1.6 ±0.1
Half-life (h)	3.6 ±0.8

^aData is the mean value ±standard deviation

AVERAGE IV CONCENTRATIONS FOR 3.95MG/KG ORAL DOSE OF TELMISARTAN 6, IN SPRAGUE-DAWLEY RATS

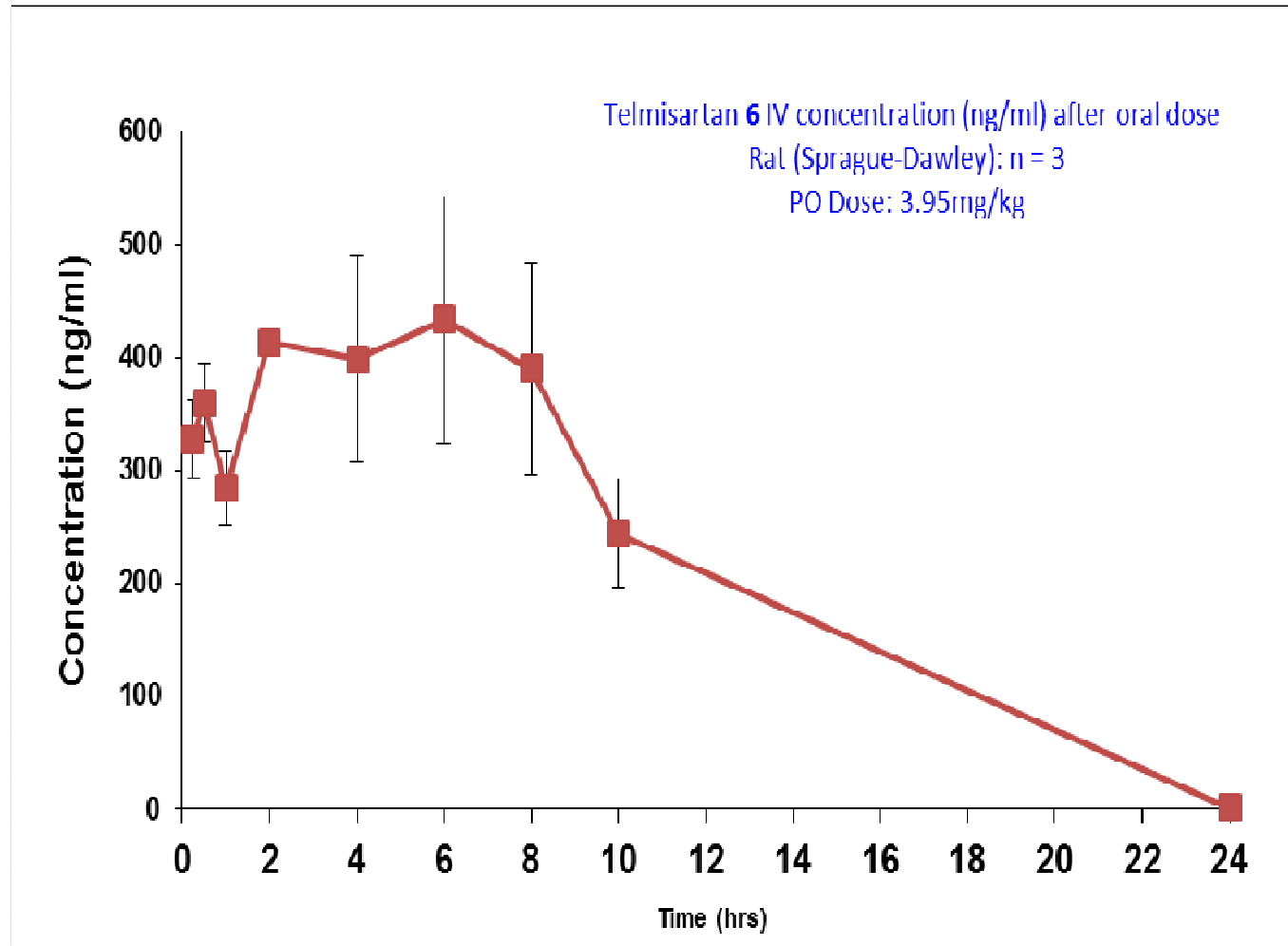


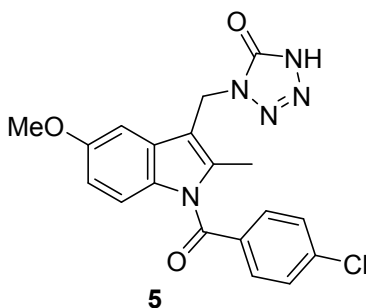
Table S4. Rat pharmacokinetics for Telmisartan 6, when dosed by oral route

Compound	6
p.o.	
dose (mg/kg)	3.95
C _{max} (ng/ml)	506 ±158
AUC _{0-24h} (ng/ml/h)	5460 ±1860
AUC _{inf} (ng/ml/h)	5480 ±1860
F (%)	59

^aData is the mean value ±standard deviation

The oral parameters for Telmisartan **6** are in agreement with literature studies. Enterohepatic recirculation is observed for this compound, which leads to a prolonged plateau (2-8hr in above).

LC DATA FOR 1-((1-(4-CHLOROBENZOYL)-5-METHOXY-1H-INDOL-3-YL)METHYL)-1,4-DIHYDRO-5H-TETRAZOL-5-ONE 5



Openlynx Report -

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File:MD1856-108

Zed:

Date:26-Jun-2014

Method:10min

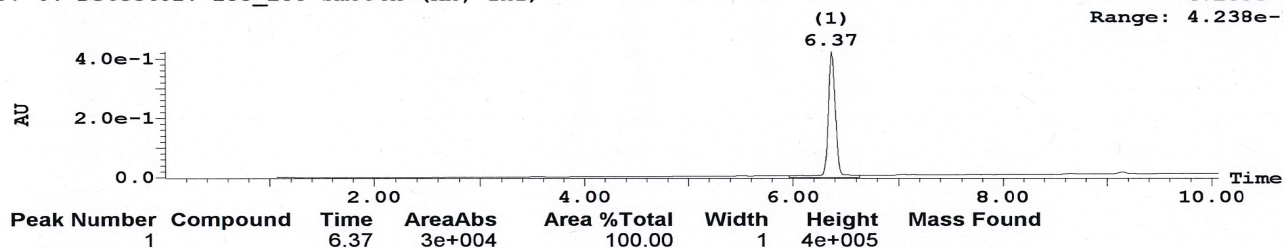
Page 1

Printed: Thu Jun 26 15:30:40 2014

3: UV Detector: 253_255 Smooth (Mn, 1x1)

4.238e-1

Range: 4.238e-1



LC DATA FOR COMMERCIAL INDOMETHACIN 4 (AS A COMPARISON TO COMPOUND 5 ABOVE)

Openlynx Report -

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Time:16:39:46

File:MD18560-114

Zed:

Date:26-Jun-2014

Method:10min

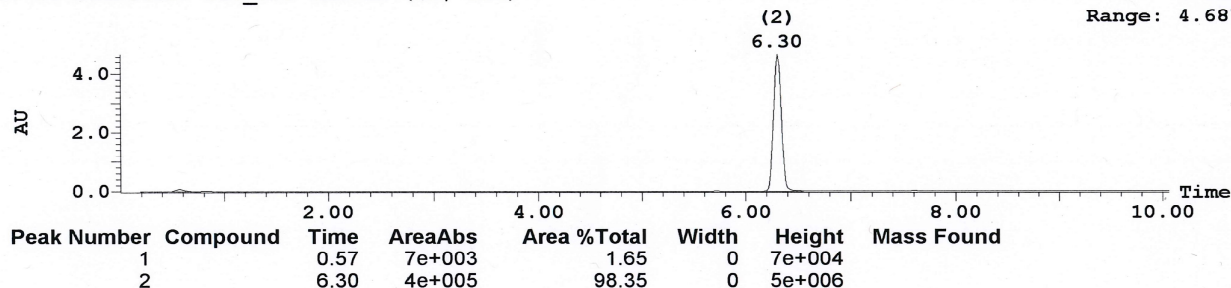
Page 1

Printed: Thu Jun 26 16:53:05 2014

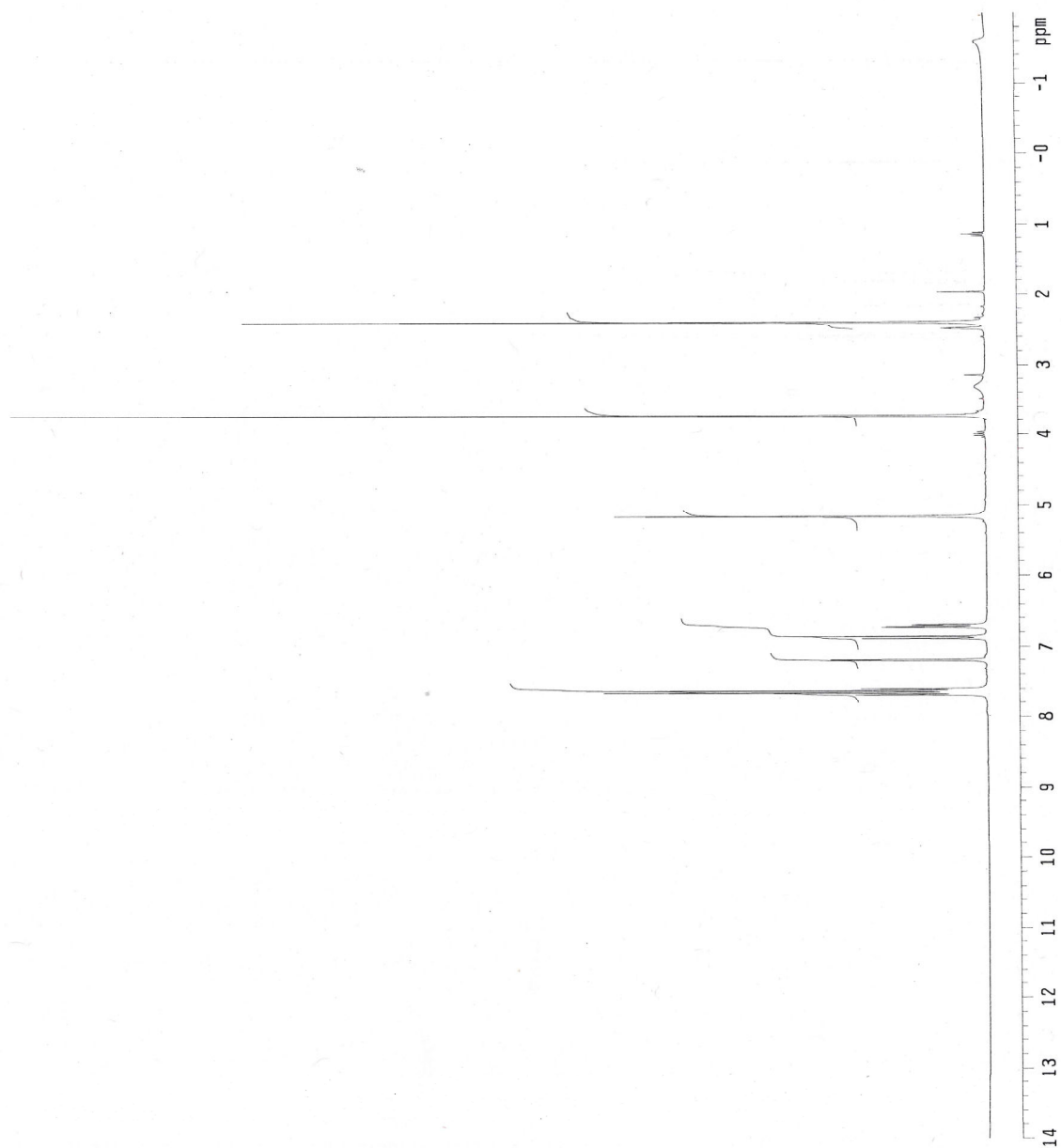
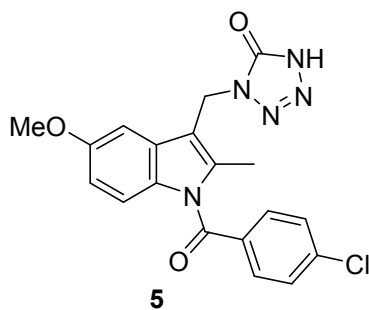
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4.687

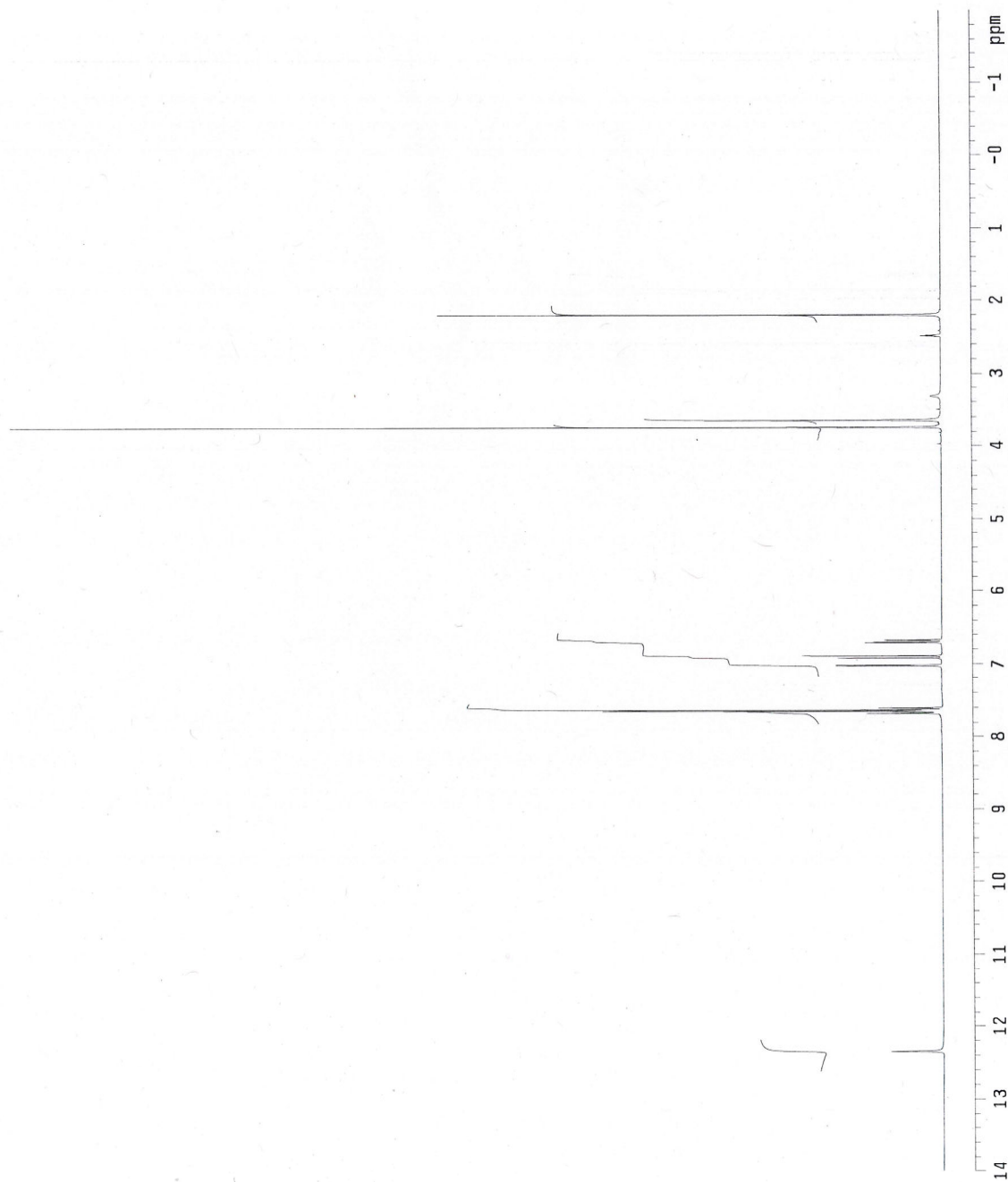
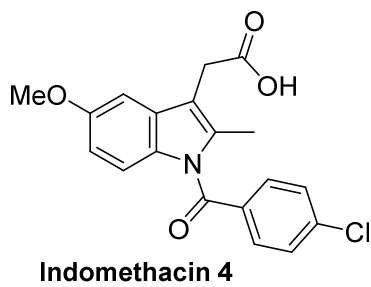
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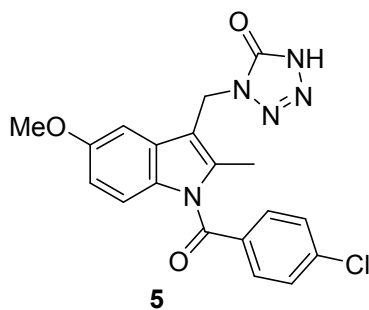
¹H NMR FOR 1-((1-(4-CHLOROBENZOYL)-5-METHOXY-1*H*-INDOL-3-YL)METHYL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 5



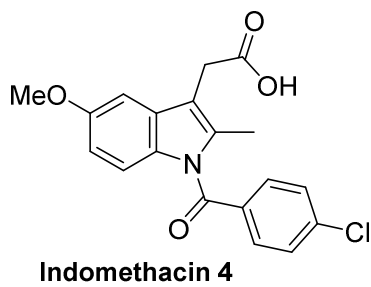
¹H NMR FOR COMMERCIAL INDOMETHACIN 4 (AS A COMPARISON TO COMPOUND 5 ABOVE)



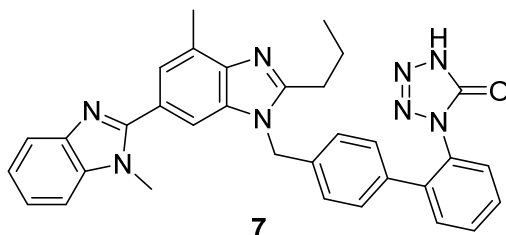
¹³C NMR FOR 1-((1-(4-CHLOROBENZOYL)-5-METHOXY-1*H*-INDOL-3-YL)METHYL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 5



^{13}C NMR FOR COMMERCIAL INDOMETHACIN 4 (AS A COMPARISON TO COMPOUND 5 ABOVE)



LC DATA FOR 1-(4'-((1,7-DIMETHYL-2'-PROPYL-1*H*,3'*H*-[2,5'-DIBENZO[*D*]IMIDAZOL]-3'-YL)METHYL-[1,1'-BIPHENYL-2-YL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 7



Openlynx Report -

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File:MD1856-098

Zed:

Date:23-Jun-2014

Method:10min

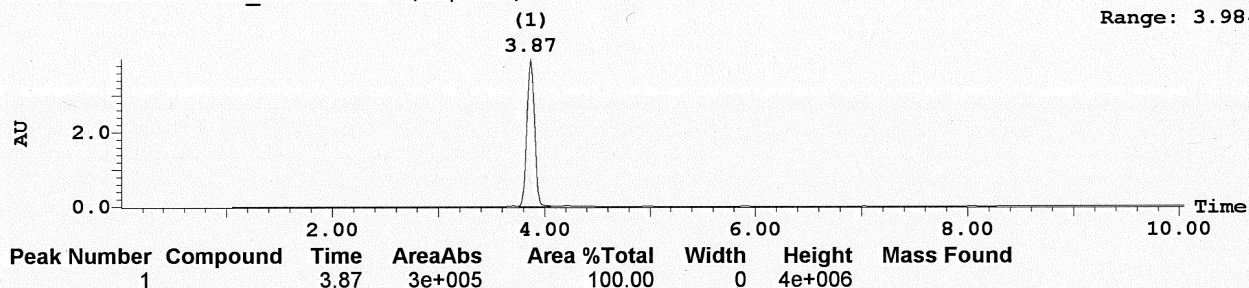
Page 1

Printed: Mon Jun 23 11:53:07 2014

3: UV Detector: 253_255 Smooth (Mn, 1x1)

3.984

Range: 3.984



LC DATA FOR COMMERCIAL TELMISARTAN 6 (AS A COMPARISON TO COMPOUND 7 ABOVE)

Openlynx Report -

Vial:1:G,7

Time:10:36:05

File:MD1856-047

Zed:

Date:26-Feb-2014

Method:10min

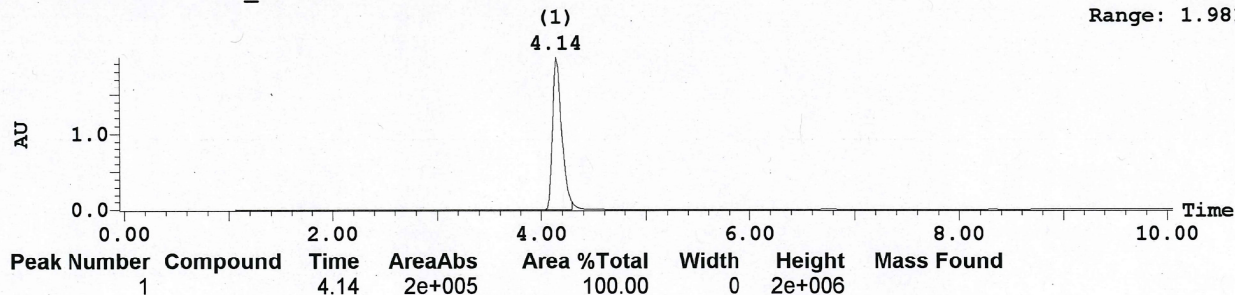
Page 1

Printed: Wed Feb 26 10:49:22 2014

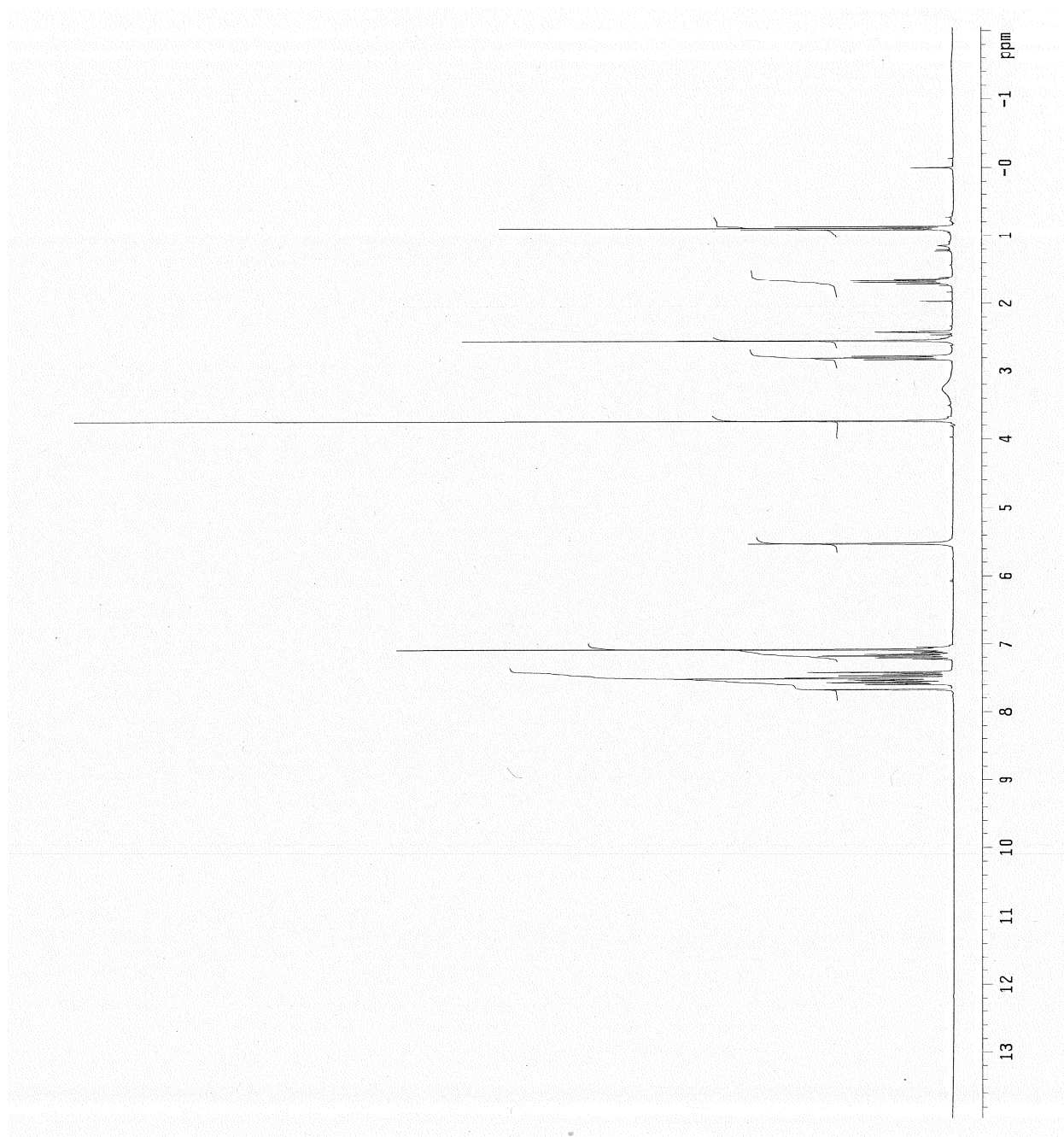
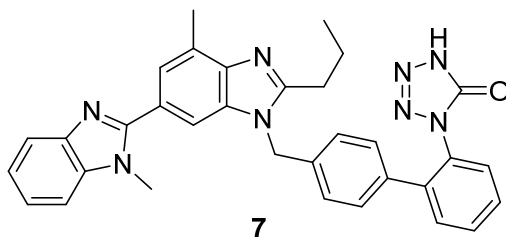
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1.981

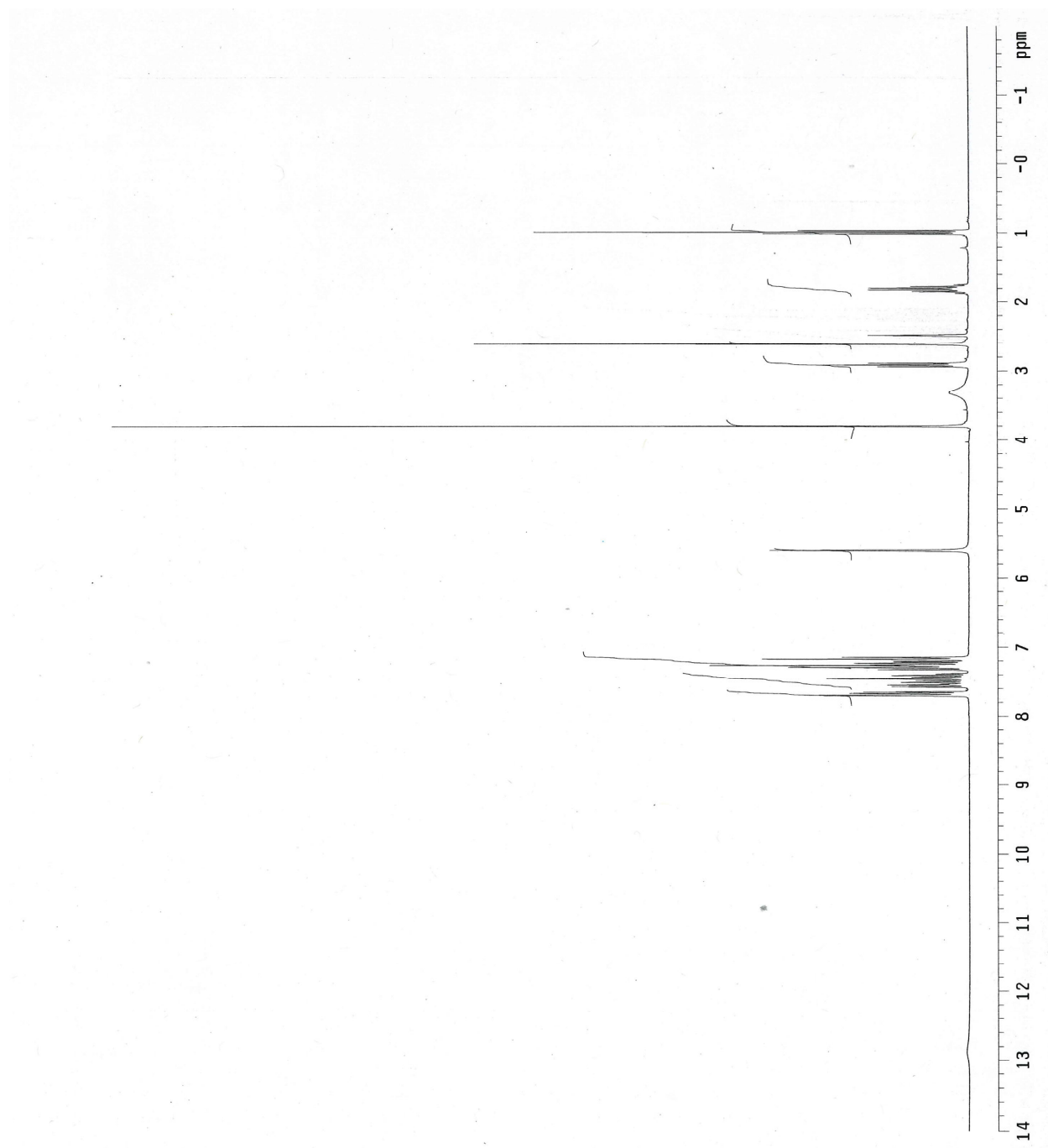
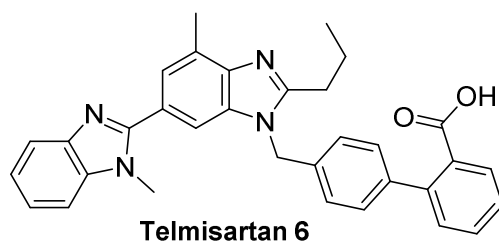
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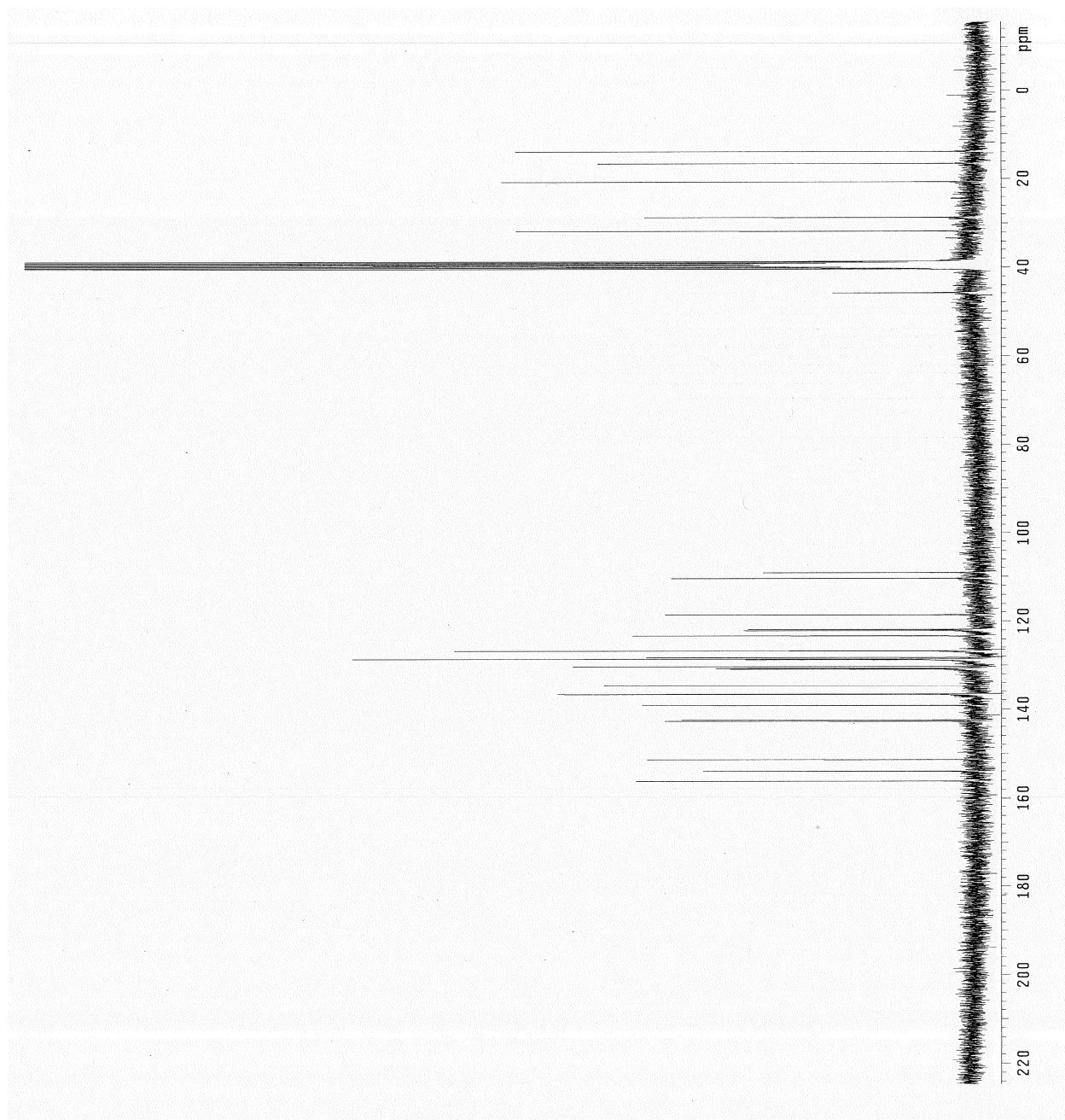
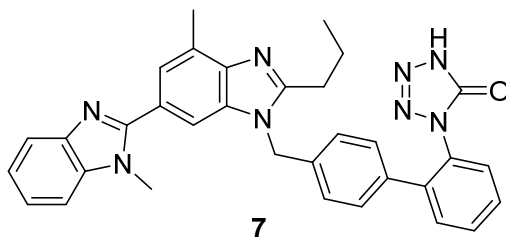
¹H NMR FOR 1-(4'-((1,7-DIMETHYL-2'-PROPYL-1*H*,3'*H*-[2,5'-DIBENZO[*D*]IMIDAZOL]-3'-YL)METHYL-[1,1'-BIPHENYL-2-YL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 7



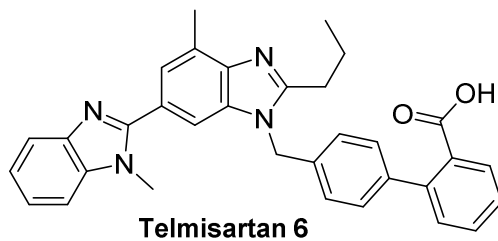
¹H NMR FOR COMMERCIAL TELMISARTAN 6 (AS A COMPARISON TO COMPOUND 7 ABOVE)



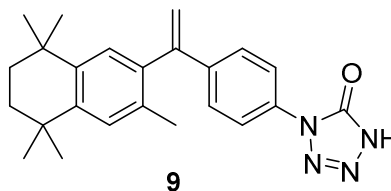
¹³C NMR FOR 1-(4'-((1,7-DIMETHYL-2'-PROPYL-1*H*,3'*H*-[2,5'-DIBENZO[*D*]IMIDAZOL]-3'-YL)METHYL-[1,1'-BIPHENYL-2-YL)-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE 7



^{13}C NMR FOR COMMERCIAL TELMISARTAN 6 (AS A COMPARISON TO COMPOUND 7 ABOVE)



LC DATA FOR 1-(4-(1-(3,5,5,8,8-PENTAMETHYL-5,6,7,8-TETRAHYDRONAPHTHALEN-2-YL)VINYL)PHENYL)-1,4-DIHYDRO-5H-TETRAZOL-5-ONE **9**



Openlynx Report -

Page 1

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File:MD1856-097

Date:02-Jun-2014

Time:15:38:35

Zed:

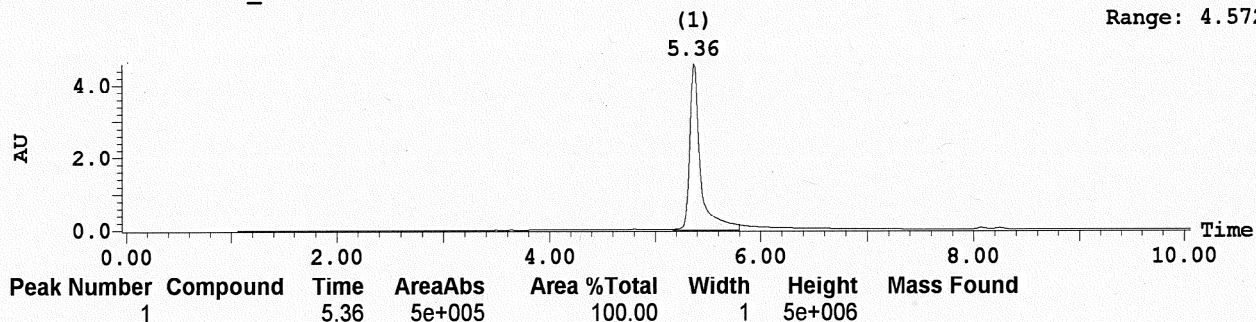
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Printed: Mon Jun 02 15:51:36 2014

3: UV Detector: 253_255 Smooth (Mn, 1x1)

4.573

Range: 4.572



LC DATA FOR COMMERCIAL BEXAROTENE **8** (AS A COMPARISON TO COMPOUND **9** ABOVE)

Openlynx Report -

Page 1

Vial:2:B,3

File:MD1856-104 Bexarotene

Date:13-Jun-2014

Time:15:42:16

Zed:

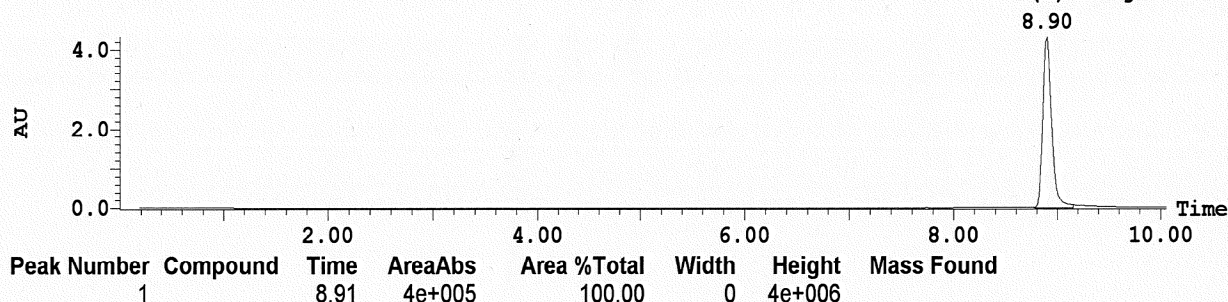
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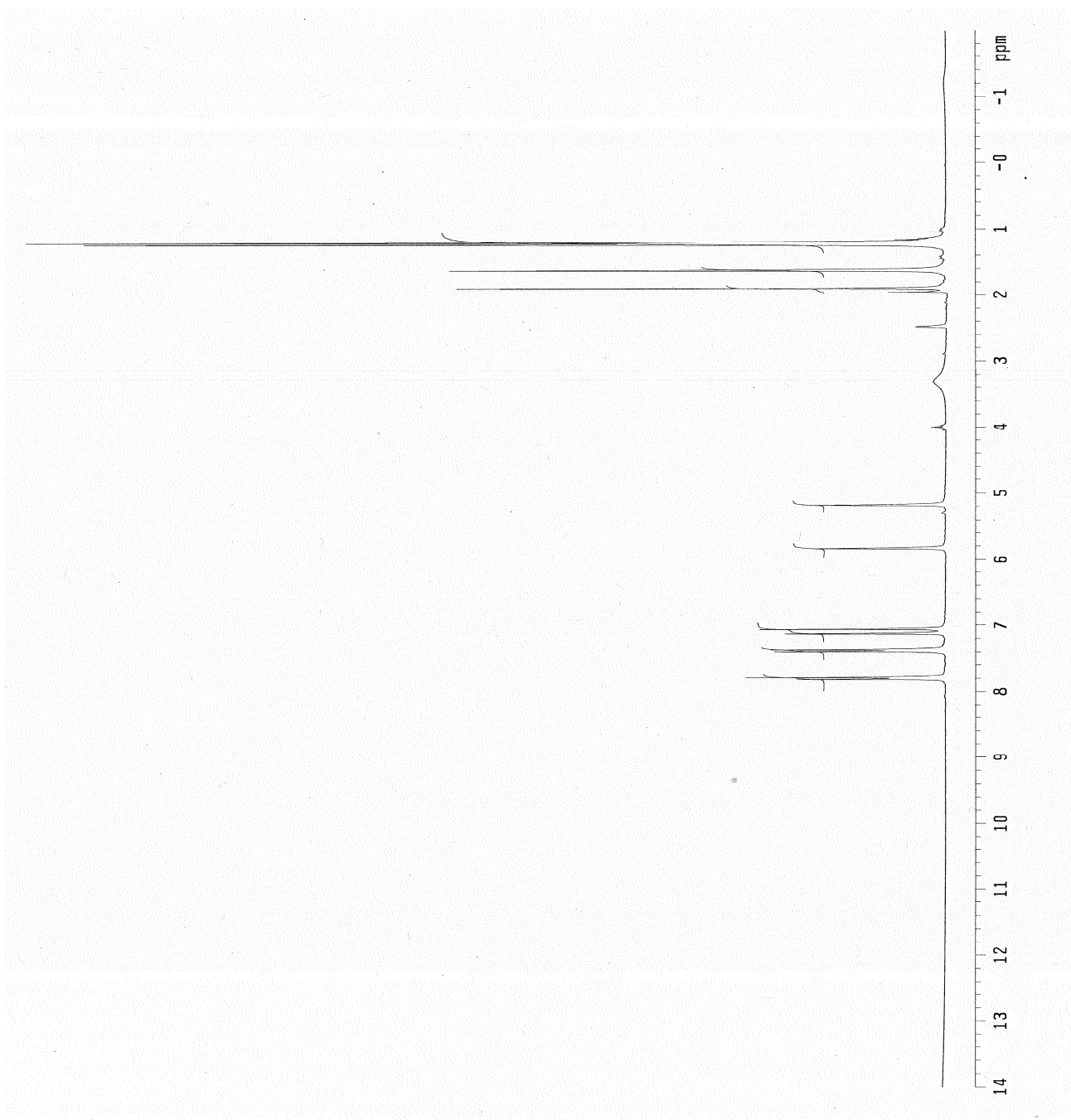
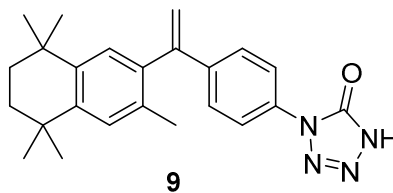
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4.326

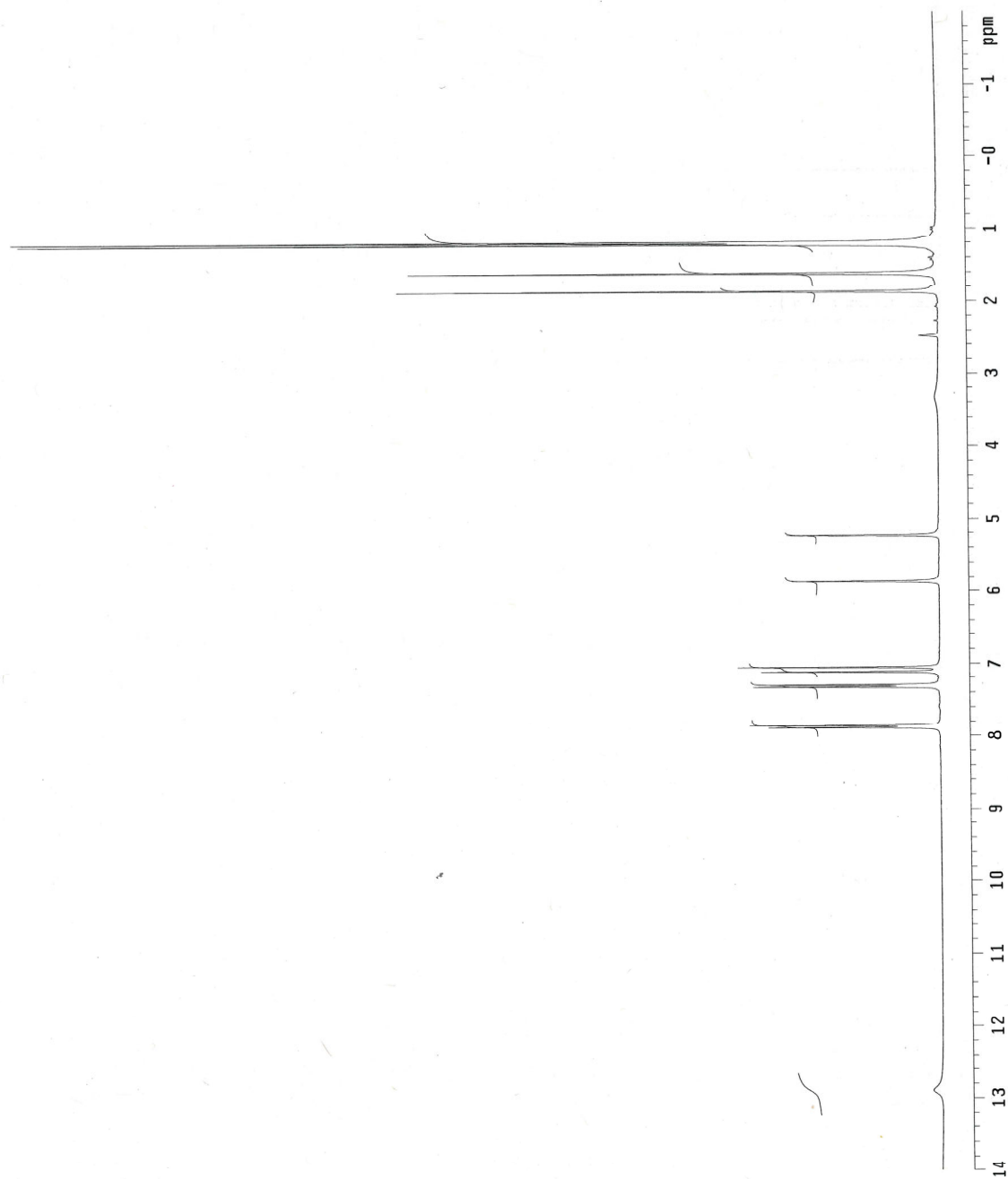
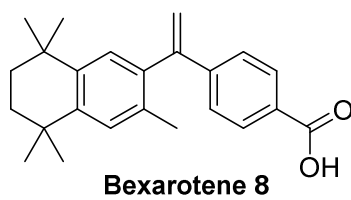
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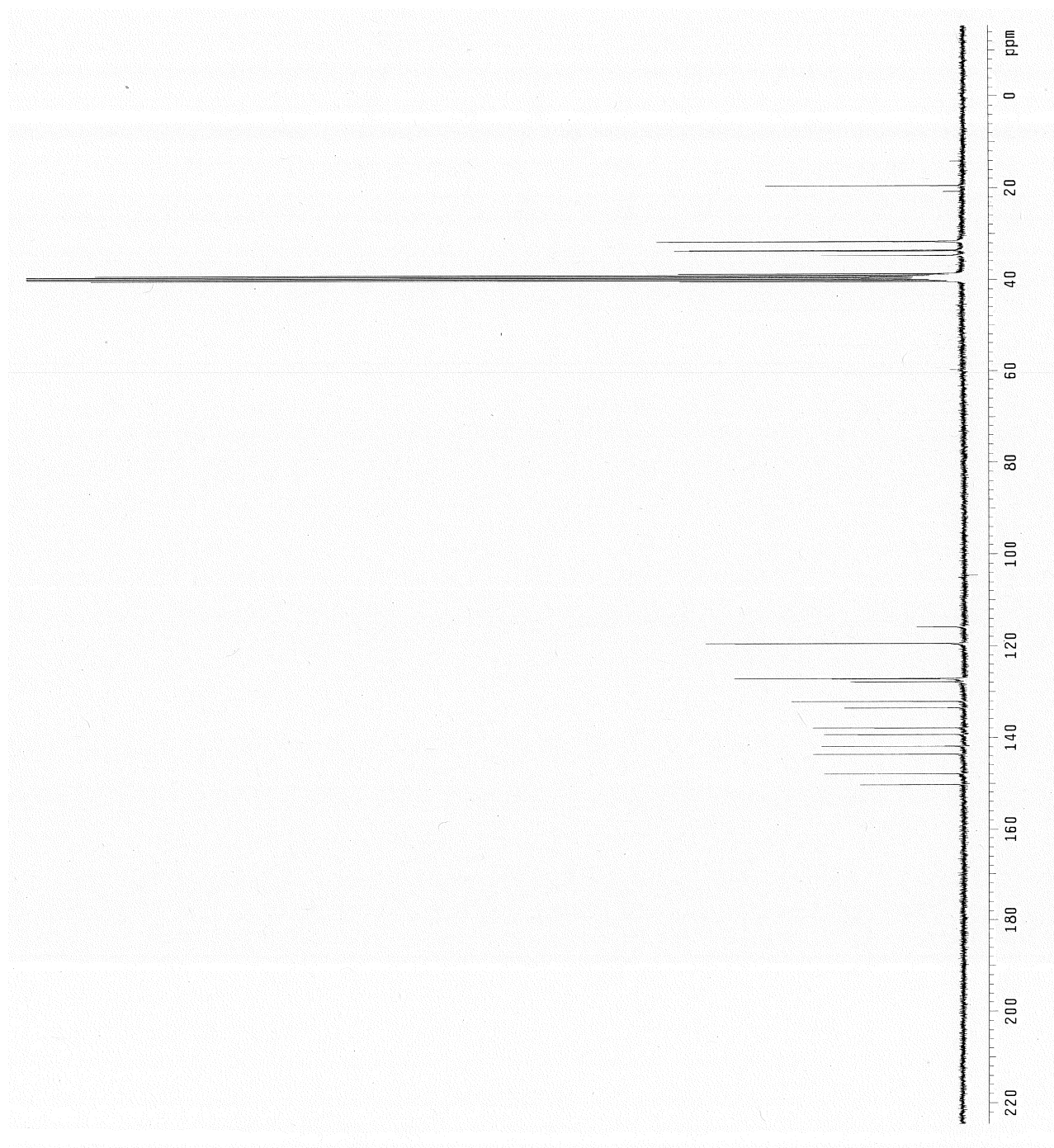
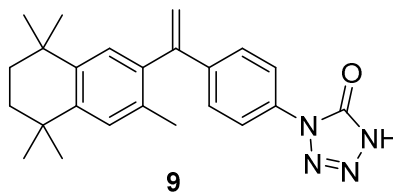
¹H NMR FOR 1-(4-(1-(3,5,5,8,8-PENTAMETHYL-5,6,7,8-TETRAHYDRONAPHTHALEN-2-YL)VINYL)PHENYL)-1,4-DIHYDRO-5H-TETRAZOL-5-ONE **9**



^1H NMR FOR COMMERCIAL BEXAROTENE (AS A COMPARISON TO COMPOUND 6U ABOVE)



¹³C NMR FOR 1-(4-(1-(3,5,5,8,8-PENTAMETHYL-5,6,7,8-TETRAHYDRONAPHTHALEN-2-YL)VINYL)PHENYL)-1,4-DIHYDRO-5H-TETRAZOL-5-ONE 9



^{13}C NMR FOR COMMERCIAL BEXAROTENE 8 (AS A COMPARISON TO COMPOUND 9 ABOVE)

