

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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INTAS PHARMACEUTICALS LTD.,

Petitioner

v.

ATOSSA THERAPEUTICS, INC.,

Patent Owner

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Case PGR2023-00043

Patent No. 11,572,334

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**EXPERT DECLARATION OF RON BIHOVSKY, Ph.D.**

(Z)-Endoxifen can be accomplished via recrystallization. Additionally, as discussed above, Ahmad's teaching that chromatography can be employed for this separation was also well within the skill of a POSA, as exemplified by Fauq. *See* Ex. 1022. In sum, the prior art, such as Liu and Fauq, demonstrate that achieving greater than 90% (Z)-Endoxifen as taught by Ahmad would have been well within the skill of a POSA. More specifically, it would have been routine for a POSA to purify the Endoxifen product synthesized according to Ahmad according to the procedures taught by Liu (crystallization) or Fauq (chromatography) to achieve greater than 90% (Z)-Endoxifen. Other approaches were also known to a POSA and also would have been routine. For example, it similarly would have been routine for a POSA to follow Liu's or Fauq's synthesis and purification process in its entirety to achieve greater than 90% (Z)-Endoxifen. My own experiments prove that this is true (Section VII.C.).

### **C. Laboratory Testing of Methods Taught by Liu**

36. The following describes my laboratory testing of methods taught by Liu. While I am a very experienced synthetic organic chemist, in my opinion, carrying out Liu's teachings would have been routine and well within the skill of a POSA as Dr. Davies has defined it (and even someone with much lower skill would have been able to follow Liu and synthesize 90% (Z)-Endoxifen). In other words, it took no extraordinary skill to carry out Liu's teachings.

**1. Synthesis of a mixture of (E)- and (Z)-Endoxifen as taught by Liu**

37. I reproduced methods of synthesis and purification disclosed by Liu.

As discussed above, Liu reports synthesis of a 51:47 mixture of (Z)- and (E)-Endoxifen and several methods for purification of (Z)-Endoxifen. I reproduced the following methods as taught by Liu: (i) synthesis followed by sequential recrystallizations as well as (ii) synthesis followed by isomerization and sequential recrystallizations. These approaches, consistent with Liu's teachings, afforded (Z)-Endoxifen with an isomeric purity of 100% and 93%, respectively. I describe in detail below the experimental protocols I followed as well as the results of those experiments.

38. Throughout the course of these experiments, I employed various analytical tools. Thin layer chromatography ("TLC") was employed to monitor reaction progress throughout the synthesis using silica gel plates. Endoxifen was eluted with a DCM:MeOH:NH<sub>4</sub>OH (90:9:1) solvent system. The plates were visualized by UV at 254 nm. No separation of (E)- and (Z)-Endoxifen was observed by TLC. Nuclear Magnetic Resonance ("NMR") spectroscopy (500 MHz) was used to confirm the structures of the chemical intermediates that I synthesized as well as (E)- and (Z)-Endoxifen. NMR spectra were obtained in dimethyl sulfoxide-d<sub>6</sub> ("DMSO-d<sub>6</sub>"). NMR spectra of (E)- and (Z)-Endoxifen were consistent with data reported by Liu (Ex. 1004 at [0076]) as well as other literature.

See Ex. 1025 (supplement to Ex. 1019), Figure S2, S4; Ex. 2011 at note 10, Ex. 1023 (supplement to Ex. 1022) at S8; Elkins, J. Pharmaceutical and Biomedical Analysis, “Characterization of the isomeric configuration and impurities of (Z)-endoxifen by 2D NMR, high resolution LC MS, and quantitative HPLC”, p. 174 – 179 (2014) (Ex. 2002). I also utilized NMR to determine the ratios of the (Z)- to (E)-Endoxifen through integration of NMR signals at  $\delta$  3.85 and 4.01 ppm respectively. (See above.) My NMR data are attached as Ex. 1024. First, I synthesized a mixture of (E)- and (Z)-Endoxifen according to the method taught by Liu. More specifically, this synthesis was conducted following the experimental protocols described in Examples 2-4 of Liu. Ex. 1004 at [0062]-[0072].

39. I obtained the product (7.82 g, Notebook number 15-27-1) in 82% yield as a pink foam which became solid after drying in vacuo followed by several days at ambient temperature. Thin layer chromatography (“TLC”) indicated an inseparable mixture of (Z)- and (E)-Endoxifen and several minor impurities. NMR indicated a 48:52 ratio of (Z)- to (E)-Endoxifen. The NMR spectrum for this product was consistent with the NMR data reported by Liu at paragraph [0071]. The NMR spectrum for the Endoxifen mixture I prepared was also consistent with NMR data reported in the literature. (See above.) The NMR spectra for the compounds I synthesized according to Liu’s procedures are attached to this declaration as Exhibit 1024.

**2. Sequential recrystallizations of a mixture of (E)- and (Z)-Endoxifen to produce highly pure (Z)-Endoxifen as taught by Liu**

40. Following synthesis of the mixture of (E)- and (Z)-Endoxifen as taught by Liu, I conducted a recrystallization of that mixture from acetone as taught by Liu. Ex. 1004 at [0070]. To that end, I dissolved the crude 48:52 mixture of (Z)- and (E)-Endoxifen (1.544 g, 15-27-1 and 15-27-2) in refluxing acetone (4.20 mL). *Id.* The crude mixture was then slowly cooled to 20 °C for 19 h, then kept for 4 days at -10 °C. The cold mixture was quickly centrifuged to isolate the solid precipitate.<sup>2</sup> *Id.* I evaporated the residual solvent in the precipitate with a stream of nitrogen, then dried in vacuo to afford a light pink solid (771 mg, 15-33-1P). NMR indicated a 33:67 ratio of (Z)- to (E)-Endoxifen. *Id.* at [0071].

41. I then dissolved this precipitate (761 mg, 15-33-1P) in refluxing isopropyl acetate (“iPrOAc”) (7.61 mL) with stirring for 1 hour, then cooled slowly and stored at 23 °C for 6 hours. *Id.* at [0072]. After vacuum filtration, the precipitate was rinsed with iPrOAc (0.2 mL) and dried in air to afford very light pink crystals (238 mg, 15-34-1P). *Id.* NMR indicated this precipitate consisted of a

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<sup>2</sup> I note that centrifugation is a minor deviation from Liu’s procedure, which isolated the solid via filtration. Centrifugation was simply a more practical approach given the scale of the recrystallization I conducted in my laboratory. In any case, filtration and centrifugation are both well-known techniques for isolating solids, and have been well-known long before September 11, 2017.

5:95 ratio of (Z)- to (E)-Endoxifen. Evaporation of the filtrate in vacuo afforded a pink solid (418 mg, 15-34-1S), which according to NMR had a 67:33 ratio of (Z)- to (E)-Endoxifen.

42. This dried filtrate was then subjected to recrystallization as taught by Liu's Example 7. Ex. 1004 at [0076]-[0077]. I dissolved this dried filtrate (416 mg, 15-34-1S) in refluxing acetone (4.1 mL) with stirring, then cooled and stirred for 40 hours at 4 °C. At this stage, a small amount of precipitate was observed. The flask was scratched to increase crystallization and stirred at 4 °C for 24 more hours. The precipitate was collected by vacuum filtration, rinsed with cold acetone, and dried to afford a white solid (149 mg, 15-35-1P). NMR indicated a 94:6 ratio of (Z)- to (E)-Endoxifen. This precipitate (147 mg, 15-35-1P) was then dissolved in refluxing acetone (2.2 mL), filtered to remove a slight precipitate, then cooled and stirred for 50 hours at 4 °C. The precipitate was collected by vacuum filtration, rinsed with cold acetone, and dried to afford a light beige solid precipitate (91 mg, 15-35-2P). NMR of this precipitate indicated a 100:0 ratio of (Z)- to (E)-Endoxifen. (Z)-Endoxifen: <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>): δ 0.84 (t, 3H), 2.27 (s, 3H), 2.40 (q, 2 H), 2.72 (t, 2H), 3.85 (t, 2H), 6.58 (d, 2H), 6.70 (d, 2H), 6.75 (d, 2H), 6.98 (d, 2H), 7.09 (m, 3H), 7.17 (m, 2H), 9.42 (br s, 1H).

43. Thus, my experiments confirm Liu's teaching of the synthesis of a mixture of (E)- and (Z)-Endoxifen followed by sequential recrystallizations which afforded (Z)-Endoxifen with 100% isomeric purity.

**3. Isomerization of (E)-Endoxifen followed by sequential recrystallizations to produce highly pure (Z)-Endoxifen as taught by Liu**

44. As I discuss above, Liu also teaches a process whereby (Z)-Endoxifen can be prepared via isomerization of (E)-Endoxifen (or a mixture of (E)- and (Z)-Endoxifen) followed by recrystallizations. I have also reproduced that process, and I have confirmed that it provides greater than 90% (Z)-Endoxifen.

45. As described above, I prepared a 48:52 mixture of (Z)- and (E)-Endoxifen according to the method taught by Liu. *See* Ex. 1004 at Examples 2-4 ([0062]-[0071]). I recrystallized this crude mixture (15-27-1 and 15-27-2) from iPrOAc to provide a precipitate enriched in (E)-Endoxifen and a filtrate enriched in (Z)-Endoxifen. Ex. 1004 at [0072]. As discussed above in Paragraph 42, I subjected the filtrate to recrystallization as taught by Liu's Example 7 to produce a 100:0 ratio of (Z)- to (E)-Endoxifen (i.e., 100% (Z)-Endoxifen as confirmed by NMR). The crystalline precipitate enriched in (E)-Endoxifen provided from Liu's process taught in [0072] was subjected to the isomerization procedure taught by Liu's Example 6. Ex. 1004 at [0073]-[0075].

46. To that end, I dissolved the Endoxifen precipitate from iPrOAc enriched in (E)-Endoxifen (236 mg, Z:E ratio of 5:95, 15-34-1P) in refluxing iPrOAc (2.36 mL) for 4 hours.<sup>3</sup> The mixture was cooled slowly and kept at 15 °C for 2 days. The precipitate was collected by vacuum filtration, rinsed with iPrOAc (0.2 mL) at 15 °C, and dried in vacuo at 35 °C to afford white crystals (182 mg, 15-34-2P). NMR indicated a 5:95 ratio of (Z)- to (E)-Endoxifen. (E)-Endoxifen <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>): δ 0.84 (t, 3H), 2.34 (s, 3H), 2.39 (q, 2 H), 2.82 (t, 2H), 4.01 (t, 2H), 6.40 (d, 2H), 6.59 (d, 2H), 6.93 (d, 2H), 7.08 (m, 5H), 7.15 (m, 2H), 9.16 (br s, 1H). The filtrate was evaporated in vacuo to afford a colorless glass (58 mg, 15-34-2S). NMR indicated a 69:31 ratio of (Z) to (E)-Endoxifen.

47. The above isomerization procedure was repeated two times as taught by Liu. Ex. 1004 at [0074] (“The above operations were repeated twice with the residual E-isomer crystals in the reactor to provide two additional crops of Z-enriched material.”) Thus, I isomerized the above Endoxifen precipitate from iPrOAc enriched in the (E)-isomer (182 mg, (Z)- to (E)-ratio 5:95, 15-34-2P) by refluxing in iPrOAc as above. The precipitate afforded white crystals (148 mg, 15-34-3P). NMR indicated a 3:97 ratio of (Z)- to (E)-Endoxifen. The filtrate afforded

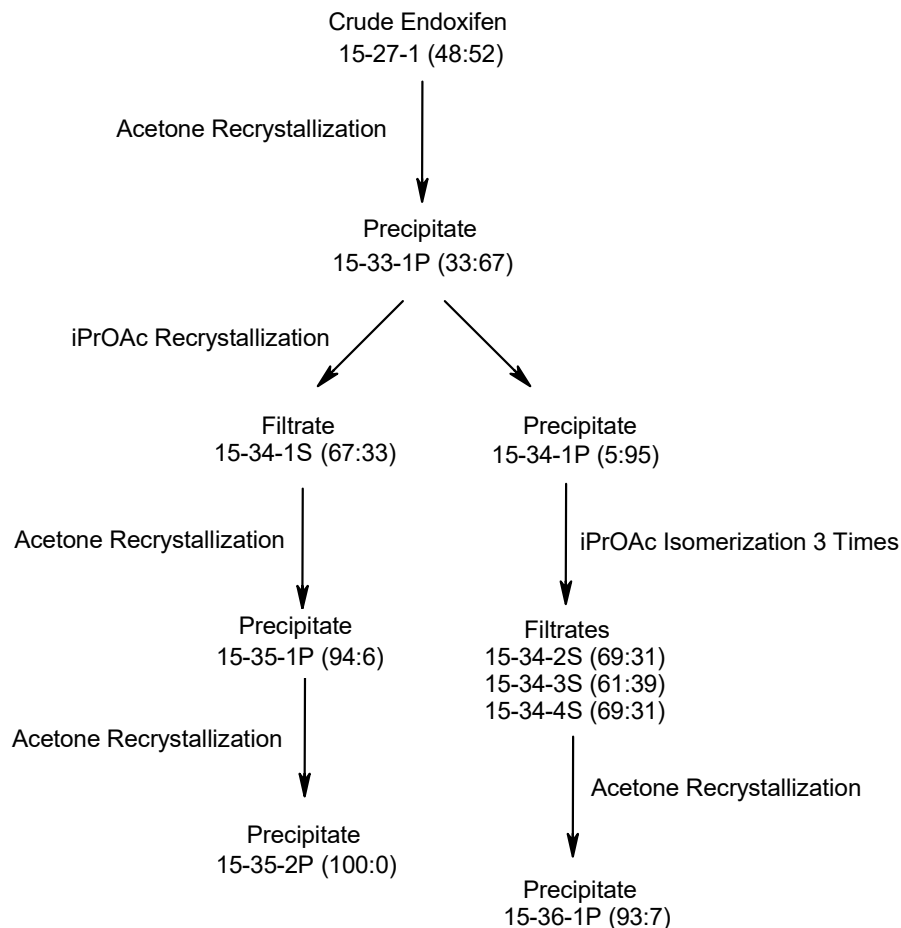
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<sup>3</sup> Liu disclosed that (E)-Endoxifen can be isomerized by heating in solvents for 0.5 to 4 hours. Ex. 1004 at [0046].

a colorless glass (34 mg, 15-34-3S) that had a 61:39 ratio of (Z)- to (E)-Endoxifen as indicated by NMR. I again subjected the precipitate from iPrOAc (146 mg, 15-34-2P) to the isomerization procedure by refluxing in isopropyl acetate as above then cooling to 15 °C for 1 day. The precipitate afforded white crystals (95 mg, 15-34-4P). NMR indicated a 3:97 ratio of (Z)- to (E)-Endoxifen. The filtrate afforded a colorless glass (50 mg, 15-34-4S). NMR indicated a 69:31 ratio of (Z)- to (E)-Endoxifen.

48. I combined the material obtained from evaporation of the filtrates from two isomerization reactions, and subjected the mixture to recrystallization from acetone to produce a material further enriched in (Z)-Endoxifen as taught by Liu's Example 7. Ex. 1004 at [0076]-[0077]. As taught by Liu, I dissolved this material (108 mg, (Z)- to (E)- ratio 69:31, 15-34-2S and 15-34-4S) in refluxing acetone (1.1 mL), then cooled and stirred for 64 hours at 4 °C. No further precipitation occurred after an additional 24 hours. The precipitate was collected by vacuum filtration, rinsed with cold acetone (~0.1 mL) and dried to afford a white solid (17 mg, 15-36-1P). NMR indicated a 93:7 ratio of (Z)- to (E)-Endoxifen.

49. The purification scheme that I used, as taught by Liu, is depicted in the figure below. The notebook numbers of each sample are indicated followed by the (Z)- to (E)-Endoxifen ratio in parentheses.



50. Thus, my experiments confirm Liu's teaching of the isomerization of a mixture enriched in (E)-Endoxifen followed by sequential recrystallizations afforded (Z)-Endoxifen with 93% isomeric purity.

51. In sum, my experiments confirm that the Liu's methods work as Liu teaches and produce highly pure (Z)-Endoxifen. As such, the methods taught by Liu would enable a POSA to achieve highly pure (Z)-Endoxifen. Indeed, my experiments following Liu's procedures provided (Z)-Endoxifen with isomeric

purities of 93% (recrystallization) and 100% (isomerization followed by recrystallization).

52. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

*Ron Bihovsky* 7/16/2024

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