BIOLOGICAL EVALUATION OF POTENTIAL RETINOID X RECEPTOR-SELECTIVE AGONISTS: THE SEARCH FOR A MORE EFFECTIVE TREATMENT FOR ALZHEIMER'S DISEASE

A thesis submitted to the University of Arizona College of Medicine – Phoenix

in partial fulfillment of the requirements for the Degree of Doctor of Medicine

Zainab Khan

Class of 2021

Mentor: Dr. Peter W. Jurutka, PhD

Scholarly Project Title: Biological evaluation of potential retinoid X receptor-selective agonists: the search for a more effective treatment for Alzheimer's disease

Authors: Zainab Khan MSIV, BS[1], Pritika Shahani BS[2], Carl Wagner PhD[2], Ichiro Kaneko PhD[1], and Peter W. Jurutka PhD[1,2]

[1] University of Arizona, College of Medicine – Phoenix, Phoenix, AZ
[2]Arizona State University, School of Mathematical and Natural Sciences, Glendale, AZ

Sources of Support: This work was supported by the National Institutes of Health (R15 CA139364).

Abstract:
Alzheimer's disease (AD) is the most common form of dementia affecting millions of people; however, treatment options are currently limited. Previous studies have shown potential to slow the progression of AD by 4-[1-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-ethenyl]benzoic acid (bexarotene), an antineoplastic agent modeled after a vitamin A derivative, in animal models. Bexarotene binds to the retinoid X receptors (RXRs) and stimulates RXR homodimerization and activation of RXR target genes. It also impacts RXR heterodimerization with other nuclear receptors, including the liver-X-receptor (LXR) and retinoic acid receptor (RAR). Bexarotene's reversal of beta amyloid (Aβ) plaques in mouse models likely occurs via RXR-LXR activation and induction of apolipoprotein E (ApoE) expression. However, bexarotene has many adverse effects, including hyperlipidemia, skin toxicity, hypothyroidism, etc. The current study developed and tested novel bexarotene analogues for their ability to upregulate ApoE expression, crucial in AD neuroprotection, without producing the significant adverse effects of hyperlipidemia and skin toxicity through upregulation of sterol regulatory element-binding protein (SREBP) and activation of RAR, respectively. In order to test bexarotene analogues, luciferase assays were performed in both human colon cancer and human embryonic kidney cell lines. Two screening assays were completed to assess their ability (1) to induce RXR homodimerization through mammalian two-hybrid assays (M2H) and (2) to induce RXR responsive element DNA sequence (RXRE) based transcription. We then assessed the ability of the analogues (1) to induce ApoE expression through LXRE-based luciferase assays, while evaluating their potential for adverse effects through (2) SREBP- and (3) RARE-based reporter systems. Results from the M2H and RXRE assays revealed that our novel analogues produce a wide range of transcriptional activity. LXRE, SREBP, and RARE assays revealed similar results. Specifically, analogue A44 had significantly higher activity with LXRE ($p < 0.05$) and significantly lower activation via SREBP ($p < 0.05$) and RARE ($p < 0.05$) as compared to bexarotene. These assays revealed that our novel bexarotene analogues can potentially be more effective and potent RXR ligands than bexarotene with the capability to circumvent RAR cross-over and elevated SREBP expression, and thus the adverse effects of bexarotene. These analogues may have the potential to slow the progression of AD through increased ApoE expression without the current limitations associated with bexarotene use.

1. INTRODUCTION

1.1. Retinoid X Receptor

The retinoid X receptor (RXR) is a member of the nuclear receptor superfamily of proteins which function as transcription factors in response to certain steroids and other signaling molecules. In general, nuclear receptors are involved in regulating cell differentiation, growth, division, and metabolism. As a nuclear receptor protein, RXR contains two domains characteristic of this family of transcription factors: a DNA-binding domain and a ligand-binding domain. Binding of an endogenous ligand induces a subsequent conformational change in the receptor ligand-binding pocket leading to activation of transcription. Conformational changes in the receptor allow for the protein to release or recruit co-regulatory molecules and form a homodimer or heterodimer between nuclear receptors. This allows the transcription complex to bind to a hormone responsive element in the DNA sequence to allow for transcription to occur. For example, 9-cis retinoic acid binds to RXR which results in its homodimerization and binding to retinoid X response elements (RXREs) in the DNA to carry out its function.[1] Although RXR was first identified in 1990, the natural ligand of this protein remained controversial. Formerly, 9-cis retinoic acid was suspected to be the natural ligand for RXR; however, numerous studies could not confirm the physiological presence of this compound.[2] More recently, 9-cis-13,14-dihydroretinoic acid was identified to be an endogenous ligand for RXR.[3] Nevertheless, the natural ligand for RXR has long been thought to be one derived from retinoic acid, a derivative of vitamin A.

1.2. Bexarotene

Bexarotene is a synthetic agent modeled after 9-*cis* retinoic acid, which is derived from vitamin A. Retinoic acid plays a major role in many processes in the human body, including early

organogenesis,[4] cell differentiation,[5] immune system regulation,[6] and growth.[7] Bexarotene mimics the actions of 9-*cis* retinoic acid and binds to RXR, stimulating RXR homodimerization and activation of RXR target genes. Clinically relevant target genes that are thought to mediate the therapeutic actions of bexarotene are the genes that serve as potential tumor suppressors. As a commercially produced compound, bexarotene is FDA-approved for the treatment of cutaneous T-cell lymphoma in patients who are refractory to at least one prior systemic therapy.[8] It inhibits cancer metastasis by inducing cell differentiation, apoptosis, and through its anti-angiogenic effects. Bexarotene has also been used "off-label" to treat non-small cell lung cancer and breast cancer.[9]

1.3. Bexarotene's Adverse Effects

Despite its many benefits, bexarotene can cause numerous clinical side-effects in patients. Many of bexarotene's adverse effects occur through its nonspecific actions with RXR and its heterodimers. Nuclear receptors in the RXR subfamily include the retinoic acid receptor (RAR), thyroid hormone receptor (TR), and vitamin D receptor (VDR), which function to regulate transcription of their own target genes via heterodimerization with RXRs. RXR heterodimer complexes can be considered permissive or nonpermissive (Fig. 1). Only primary nuclear receptor ligands (e.g., vitamin D, thyroid hormone) activate nonpermissive heterodimers, while RXR-specific or partner-selective ligands activate permissive heterodimers. VDR and TR are generally nonpermissive partners for RXR, meaning the ligand of the RXR partner does not characteristically bind to its receptor when it is heterodimerized with RXR. RAR is conditionally nonpermissive. Finally, liver X receptor (LXR), peroxisome proliferator-activated receptor (PPAR), and farnesoid X receptor (FXR) are considered fully permissive.[10]

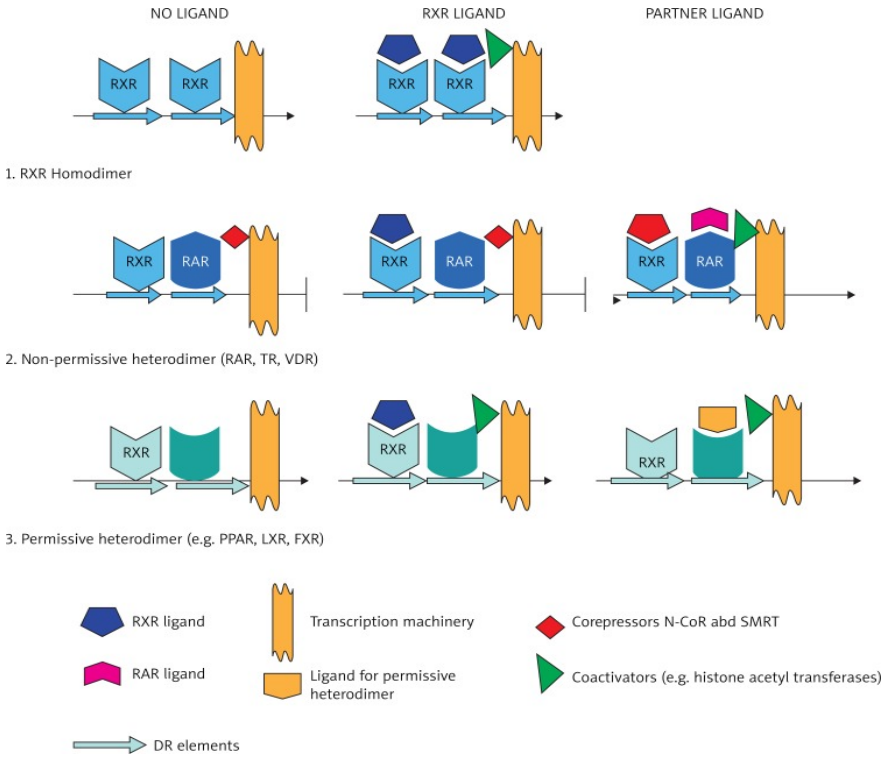

Figure 1. Molecular mechanism of the retinoid X receptor.
Figure taken from Sokołowska-Wojdyło et al. (2013)[11]

The presence of synthetic ligands with high affinity for RXR (e.g., bexarotene) can promote RXR homodimerization by diverting RXR proteins away from heterodimerizing with nonpermissive receptors and can cause permissive receptors to become activated without the partner ligand. Interruption in RXR heterodimerization, or activation of permissive heterodimers, in a variety of important biological pathways results in many of the clinical side-effects associated with bexarotene use. In one study, 79% of patients who took bexarotene presented with hypertriglyceridemia of all grades, 48% experienced hypercholesterolemia, and 40% experienced hypothyroidism.[12] Disruption of nonpermissive RXR-TR activation by bexarotene has been shown to result in hypothyroidism,[13] while stimulation of permissive RXR-RAR activity has been demonstrated to result in cutaneous toxicity and hyperplasia.[14] Furthermore, stimulation of permissive RXR-LXR activity by bexarotene has been shown to result in hyperlipidemia via

LXREs in the SREBP promoter.[15,16] Additional adverse effects of bexarotene include leucopenia, headache, and fatigue. Studies have demonstrated that RXR selective molecules can be manufactured to favor RXR activation over RAR interactions, preserving the protective response without increasing the corresponding side effects caused by RAR activation.[17]

1.4. Bexarotene and Neurodegenerative Disease

Although bexarotene has been historically used to treat cutaneous T-cell lymphoma, recent studies have shown that bexarotene may potentially function to provide neuroprotective effect in amyotrophic lateral sclerosis (ALS) and reduce amyloid plaques and improve mental functioning in Alzheimer's disease.[18,19] Bexarotene is thought to perform its neuroprotective effects via RXR-LXR heterodimers, which can potentially induce apolipoprotein E (ApoE) expression and promote appropriate processing of lipoproteins in the nervous system. In one study, bexarotene was shown to promote a protective environment for motor neurons by reduction of reactive astrogliosis and preservation of perisomatic synapsis. Early motor neuron degeneration was also shown to be delayed in the lumbosacral spinal cord by bexarotene in ALS mice.[18] In another study, bexarotene was shown to target the primary nucleation step in Aβ42 aggregation and postpone formation of toxic species and Aβ42 deposition in neuroblastoma cells.[19] Furthermore, a study performed in AD mice determined that hippocampal-dependent cognitive defects were reversed by selective modification of neuroinflammation by bexarotene.[20] However, a consensus on bexarotene's impact on neurodegenerative diseases has not been reached. Some studies have shown no significant memory improvement or plaque reduction by bexarotene in AD mice[21] or AD patients.[22]

1.5. Retinoid X Receptor Agonists

RXR agonism has been demonstrated by numerous compounds, most notably bexarotene, an acrylic acid 3-[4-Hydroxy-3-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)phenyl]

-2-propenoic acid (CD3254),[23] and 6-(ethyl(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)amino)nicotinic acid (NEt-TMN) (Fig. 2).[24] In a previous study by our group, several analogous compounds of CD3254 were synthesized and evaluated for RXR agonism.[25] These compounds displayed differential gene expression and side-effects *in vivo*.[26] Additionally, analogs of NEt-TMN have also been studied by our group and others.[26] Most recently, the Kakuta group evaluated compound A48 and A50 for RXR agonism as a potential treatment of type II diabetes.[27] In an effort to create more potent and selective RXR analogues, structural motifs from potent CD3254 analogues and rexinoids containing pyridine rings (e.g., NEt-TMN) were combined by our organic chemist, Dr. Carl Wagner, to synthesize analogues A42-A47. Furthermore, enhanced RXR agonism was observed by our group in analogues arising from the addition of fluorine atoms proximal to the carboxylic acid group of bexarotene.[26] To study this further, Dr. Wagner also synthesized analogues A49 and A51 by adding a fluorine group to analogues A48 and A50, respectively (Fig. 2). Therefore, this study assessed eight

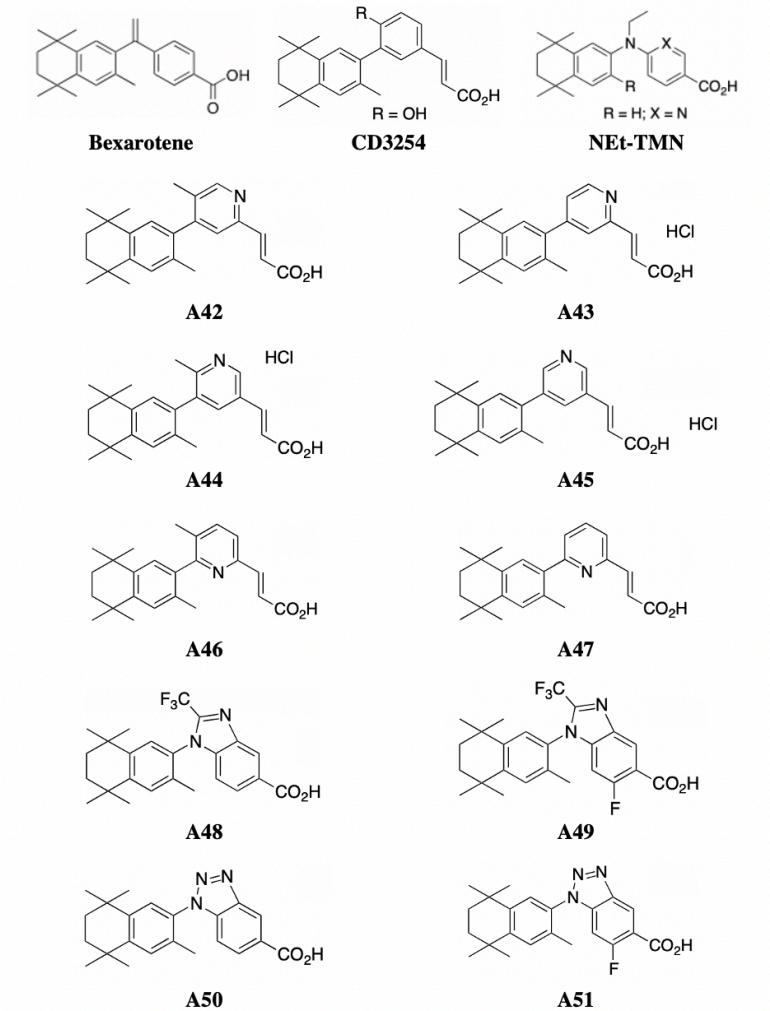

Figure 2. Structural figures of bexarotene, CD3254, Net-TMN, and rexinoids A42-A51 prepared and evaluated in the present study.

novel and two previously studied bexarotene analogues for their ability to upregulate neuroprotective ApoE expression without producing significant adverse effects by RAR and SREBP signaling typical of bexarotene in an effort to develop analogues that minimize the clinical side-effects of bexarotene and improve pharmacokinetic and pharmacodynamic properties of the drug.

2. METHODS

2.1. Mammalian Two Hybrid (M2H) Assay

The M2H assay was utilized to assess the ability of the bexarotene analogues to induce RXR homodimerization by evaluating protein-protein interaction. In a mammalian two-hybrid system, one protein is fused to a DNA binding domain (BD) while another protein is fused to an activator domain (AD). Upon exposure to ligand, if the two proteins physically interact, they will form a dimer, and this results in coactivator recruitment and the activation of the luciferase reporter gene. The activation of the luciferase gene results in the emission of light which can be measured using a luminometer. This experiment was conducted in a human colorectal carcinoma (HCT116) cell line. HCT116 cells were transfected with pCMV-AD-RXRα, pCMV-BD-RXRα, pFR-luciferase reporter gene, and a renilla control plasmid for 24 hours utilizing a liposome-mediated transfection protocol. The cells were then treated with the following 12 treatment groups with three replicates per treatment group: ethanol as a negative control, bexarotene as a positive control, and analogues A42-A51 at 100 nM for 24 hours. Luciferase activity was then measured as a function of RXR homodimerization in response to potential RXR analogues. Three independent assays were performed to determine consistency of results.

2.2. Transcriptional Luciferase Assay

Transcriptional luciferase assays were utilized to examine how effectively ligand binding

triggers transactivation via the transcription factor. When the transcription factor is activated by a ligand, it is able to recognize and bind to responsive elements present in the DNA. This leads to the induction of the luciferase reporter gene. RXRE-based transcriptional assays were conducted in the HCT116 cell line to assess the ability of bexarotene analogues to regulate RXRE-based signaling. LXRE-based transcriptional assays were performed in a human embryonic kidney (HEK293) cell line to assess the effects of bexarotene analogues on ApoE signaling. RARE- and SREBP-based transcriptional assays were also be employed to test the effects of novel bexarotene analogues on RAR and SREBP promoter activation.

2.2.1. Evaluation of RXR Activation

HCT116 cells were transfected with human RXRα (hRXRα), a RXRE-luciferase transporter gene, and a renilla control plasmid for 24 hours utilizing a liposome-mediated transfection protocol. The cells were then treated with the following 12 treatment groups with three replicates for each treatment group: ethanol as a negative control, bexarotene as a positive control, and analogues A42-A51 at 100 nM for 24 hours. Luciferase activity was then measured as a function of RXRE-based transcriptional activity in response to potential RXR analogues. Three independent assays were performed to determine consistency of results.

2.2.2. Evaluation of LXR Activation

Since ApoE expression is strongly dependent on LXR-LXRE-mediated activation, HEK293 cells were transfected with expression vectors for hRXRα, human LXRα (hLXRα), a LXRE-luciferase reporter gene, and a renilla control plasmid for 24 hours utilizing a liposome-mediated transfection protocol. The cells were then treated with the following 24 treatment groups with three replicates for each treatment group: ethanol as a negative control, LXR ligand T0901317 (T0) as a positive control, bexarotene, analogues A42-A51 or a combination of an

analogue/bexarotene and T0 to mimic endogenous conditions at 100 nM for 24 hours. LXR/LXRE-dependent activity in the presence of bexarotene analogues was compared to bexarotene. Three independent assays were performed to determine consistency of results.

2.2.3. Evaluation of SREBP Promoter Activation

HEK293 cells were transfected with an expression vector for hLXRα, an SREBP-promoter-luciferase reporter gene that contains RXR-dependent response elements, and a renilla control plasmid for 24 hours utilizing a liposome-mediated transfection protocol. The SREBP-luciferase reporter plasmid contains DNA binding sites for RXR-LXR and is thus activated by both RXR and LXR ligands. After 24 hours of transfection, cells were treated with the following 24 treatment groups with three replicates for each treatment group: ethanol as a negative control, T0 as a positive control, bexarotene, analogues A42-A51, or a combination of an analogue/bexarotene and T0 to mimic endogenous conditions at 100 nM for 24 hours. Three independent assays were performed to determine consistency of results.

2.2.4. Evaluation of RAR Agonist Activity

HEK293 cells were transfected with expression vectors for hRXRα, hRAR, a RARE-luciferase reporter gene, and a renilla control plasmid for 24 hours utilizing a liposome-mediated transfection protocol. The cells were then treated with the following 13 treatment groups with three replicates for each treatment group: ethanol as a negative control, all-trans-retinoic acid (RA) as a positive control, bexarotene, and analogues A42-A51 at 100 nM for 24 hours. RAR agonist activity was compared to bexarotene. Three independent assays were performed to determine consistency of results.

3. RESULTS

3.1. Evaluation of RXR Homodimerization

In order to assess the ability of the novel bexarotene analogues to induce RXR homodimerization, M2H assays were conducted. Our results revealed that certain bexarotene analogs were capable of binding RXR, inducing homodimerization, and regulating subsequent gene transcription. Of the bexarotene analogues assessed, A42 seemed to have greater activity than bexarotene, A43 was comparable to bexarotene, A48 had slightly lower activity than bexarotene, and the remaining analogues seemed to be less potent than bexarotene at inducing RXR homodimerization (Fig. 3).

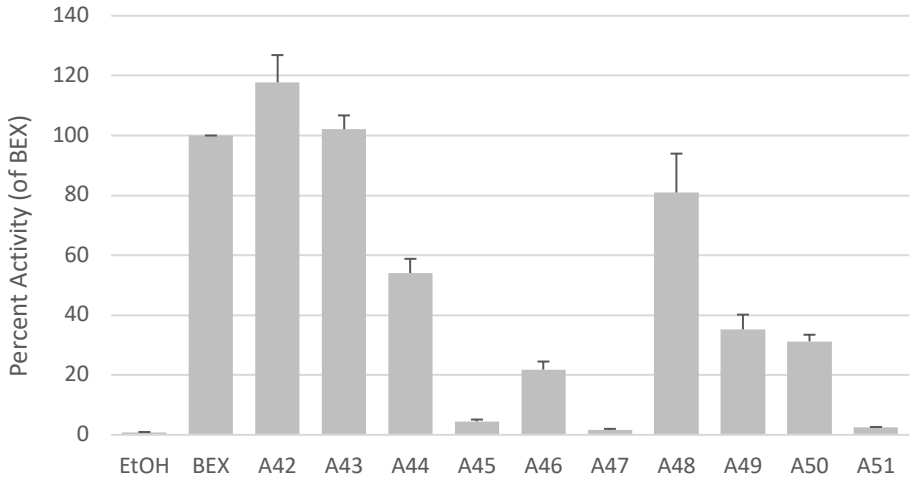

Figure 3. Evaluation of RXR Homodimerization in HCT116 cell line.

3.2. Evaluation of RXR Activation

In order to assess the ability of bexarotene analogues to induce direct RXR transactivation, transcriptional luciferase assays were performed. Unlike the M2H assays, the more specific RXRE assay revealed that A42, A43, A48 had higher activity than bexarotene, A44, A49, and A50 were comparable to bexarotene, and the remaining analogues were less potent than bexarotene (Fig. 4).

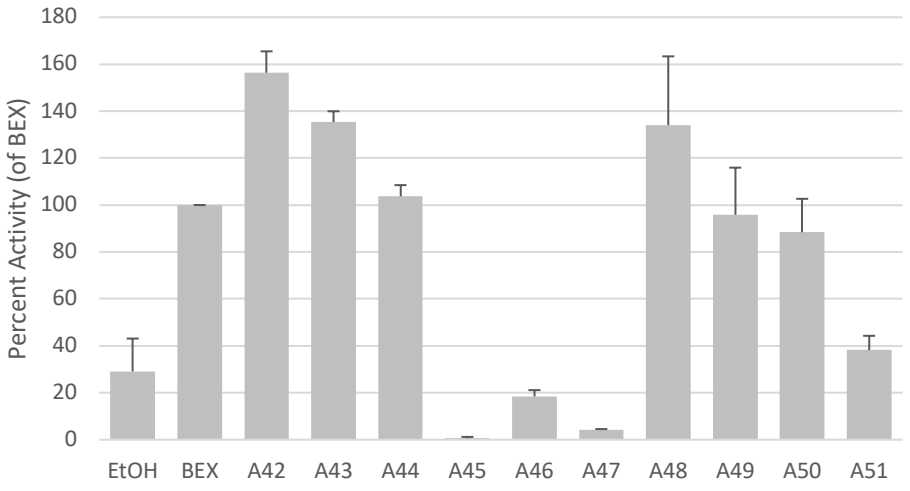

Figure 4. Evaluation of direct RXR Activation in HCT116 cell line.

3.3. Evaluation of LXR Activation

As ApoE expression is dependent on LXR activation, LXR-LXRE-based transcriptional assays were performed to assess the effects of bexarotene analogues on this signaling pathway in HEK293 cells. Bexarotene had comparable activity as T0, a synthetic LXR ligand. A44 had significantly higher activity than bexarotene ($p < 0.05$). A42, A43, A46, and A47 had comparable activity to bexarotene, and the remaining analogues had lower LXRE activation as compared to bexarotene (Fig. 5).

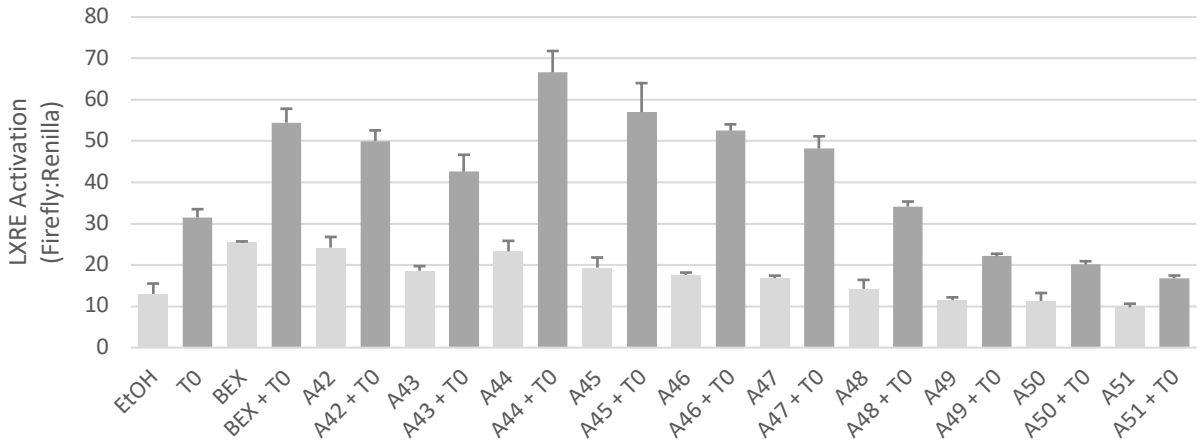

Figure 5. Evaluation of LXRE activation in HEK293 cell line.

3.4. Evaluation of SREBP Promoter Activation

In order to assess the implications bexarotene analogues use may have on SREBP-dependent hyperlipidemia like that seen with bexarotene use, SREBP-dependent transcriptional assays were performed in HEK293 cell line. A42 had comparable activity to bexarotene, A44 had significantly lower activity than bexarotene ($P < 0.05$) while the remaining analogues were also less potent than bexarotene at inducing SREBP transactivation (Fig. 6).

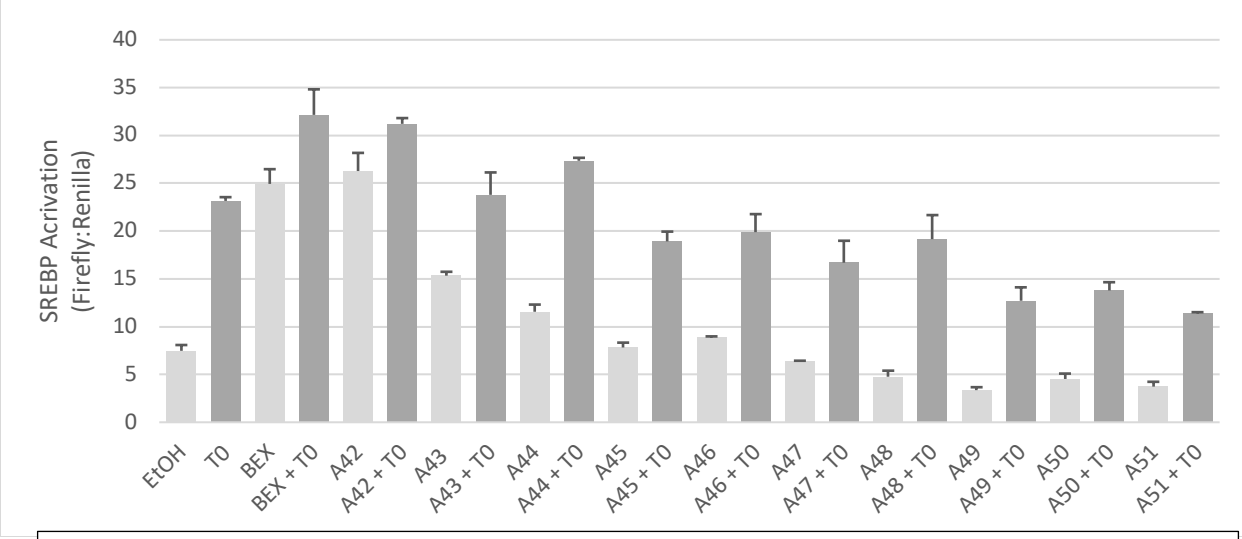

Figure 6. Evaluation of SREBP transactivation in HEK293 cell line.

3.5. Evaluation of RAR Activity

To assess the effects of bexarotene analogues on RAR activity associated with skin toxicity and hyperplasia, RARE-based transactional assays were performed in HEK293 cells. Our assays revealed A42 had higher RAR agonist activity than bexarotene while A43 was comparable to bexarotene. A44 had significantly lower activity than bexarotene ($P < 0.05$) while the remaining analogues were also less potent than bexarotene at inducing RAR transactivation associated with skin toxicity (Fig. 7).

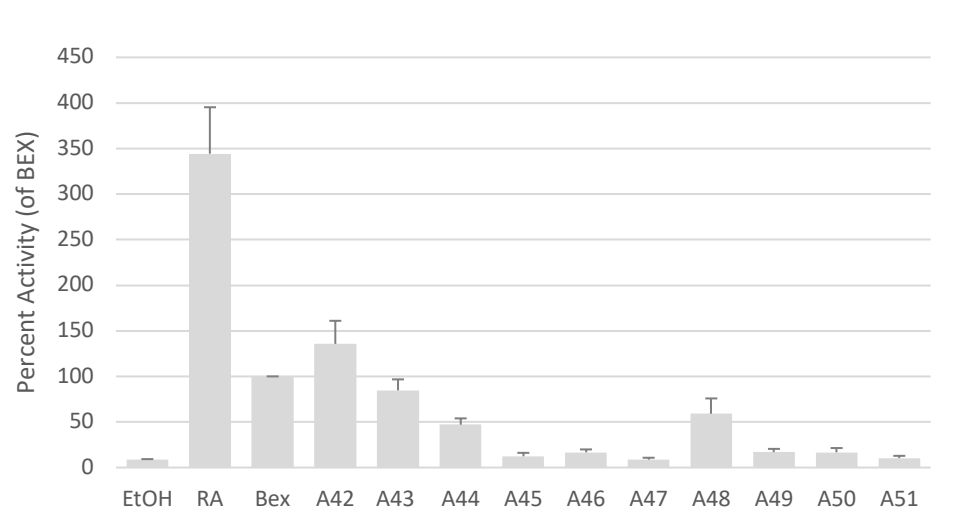

Figure 7. Evaluation of RARE activation in HEK293 cell line.

4. DISCUSSION

Bexarotene is FDA-approved for the treatment of cutaneous T-cell lymphoma; however, it has been evaluated for various different clinical implications ranging from treatment of other cancer types to treatment of SARS-CoV-2.[28] Notably, recent studies also suggest that bexarotene may be a promising contender for the treatment of AD.[19,20] Although bexarotene is an effective treatment option for cutaneous T-cell lymphoma, it is associated with a side effect profile which includes hypothyroidism,[13] cutaneous toxicity and hyperplasia,[14] and hyperlipidemia.[15,16] The experiments and resultant data presented in this thesis explore the potential for our novel bexarotene analogues to serve as RXR agonists with implications for downstream signaling through various receptors in the nuclear receptor superfamily. This novel study reveals the importance and potential to develop bexarotene analogues with the ability to be more targeted in RXR heterodimer pathway activation. Our M2H and RXRE assays illustrated that our novel analogues produce a wide range of RXR transcriptional activity with some compounds having greater activation and others having lower activation as compared to bexarotene. LXRE, SREBP, and RARE assays revealed similar results. Specifically, analogue A44 had significantly higher

activity with LXRE, which would indicate the potential for significantly greater ApoE expression crucial in neuroprotection, as compared to bexarotene ($p < 0.05$). Moreover, A44 also had significantly lower activation via SREBP ($p < 0.05$), which is associated with bexarotene-induced hyperlipidemia, and lower RARE activity ($p < 0.05$), which is associated with bexarotene-induced cutaneous toxicity, as compared to bexarotene. Thus, analogue A44 is a prime candidate for further testing because it may possess increased induction of ApoE in brain while mitigating RXR-directed hyperlipidemia and cutaneous toxicity side-effects because of its lower activity in the SREBP and RARE assays.

Further research will expand on the findings of this study and further assess the potential for our novel bexarotene analogues to serve as RXR agonists and assist in treatment of neurodegenerative diseases. More specifically, the impact of the analogues on ApoE gene expression will be evaluated through quantitative polymerase chain reaction. Furthermore, half maximal effective concentration (EC_{50}) assays will be conducted to determine the EC_{50} value for each analogue. Our assays reveal that our novel bexarotene analogues can potentially be more effective and potent RXR ligands than bexarotene with the capability to circumvent RAR cross-over and elevated SREBP expression, and thus the adverse effects of bexarotene. These analogues may have the potential to slow the progression of AD through increased ApoE expression without the current limitations associated with bexarotene use.

REFERENCES

1. Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell. 1995;81:541−550.
2. Dawson MI, Xia Z. The retinoid X receptors and their ligands. Biochim Biophys Acta. 2012;1821(1):21-56.
3. de Lera ÁR, Krezel W, Rühl R. An Endogenous Mammalian Retinoid X Receptor Ligand, At Last! ChemMedChem. 2016 May 19;11(10):1027-37.
4. Duester G. Retinoic acid synthesis and signaling during early organogenesis. *Cell*. 2008;134(6):921-931.
5. Duester G. Retinoid signaling in control of progenitor cell differentiation during mouse development. *Seminars in cell & developmental biology*. 2013;24(0).
6. Carratù M, Marasco C, Mangialardi G, Vacca A. Retinoids: novel immunomodulators and tumour-suppressive agents? *British Journal of Pharmacology*. 2012;167(3):483-492.
7. Raifen R, Altman Y, Zadik Z. Vitamin A levels and growth hormone axis. *Horm Res* 1996;46:279-281.
8. Gniadecki R, Assaf C, Bagot M, Dummer R, Duvic M, Knobler R, Ranki A, Schwandt P, Whittaker S. The optimal use of bexarotene in cutaneous T-cell lymphoma. *British Journal of Dermatology*. 2007;157:433–440.
9. Qu L, Tang X. Bexarotene: a promising anticancer agent. *Cancer Chemother Pharmacol*. 2010;65(2): 201-205.
10. Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell*. 1995;81:541−550.

11. Sokołowska-Wojdyło M, Lugowska-Umer H, Maciejewska-Radomska A. Oral retinoids and rexinoids in cutaneous T-cell lymphomas. Postepy Dermatol Alergol. 2013 Feb;30(1):19-29.
12. Duvic M, Martin AG, Kim Y, Olsen E, Wood GS, Crowley CA, Yocum RC. Phase 2 and 3 clinical trial of oral bexarotene (targretin capsules) for the treatment of refractory or persistent early-stage cutaneous t-cell lymphoma. *Arch Dermatol*. 2001;137(5):581–593.
13. Sherman SI. Gopal J. Haugen BR. Chiu AC. Whaley K. Nowlakha P. Duvic M. Central hypothyroidism associated with retinoid X receptor-selective ligands. *N. Engl. J. Med*. 1999;340:1075−1079.
14. Thacher SM, Standeven AM, Athanikar J, Kopper S, Castilleja O, Escobar M, Beard RL, Chandraratna RAS. Receptor specificity of retinoid-induced epidermal hyperplasia: effect of RXR-selective agonists and correlation with topical irritation. *J. Pharmacol. Exp. Ther.* 1997;282:528−534.
15. Murthy S. Born E. Mathur SN. Field FJ. LXR/RXR Activation Enhances Basolateral Efflux of Cholesterol in CaCo-2 Cells. *J. Lipid Res*. 2002;43:1054−1064.
16. Field FJ. Born E. Mathur SN. LXR/RXR Ligand Activation Enhances Basolateral Efflux of Betasitosterol in CaCo-2 Cells. *J. Lipid Res*. 2004;45:905−913.
17. Heck MC, Wagner CE, Shahani PH, MacNeill M, Grozic A, Darwaiz T, Shimabuku M, Deans DG, Robinson NM, Salama SH, Ziller JW, Ma N, van der Vaart A, Marshall PA, Jurutka PW. Modeling, Synthesis, and Biological Evaluation of Potential Retinoid X Receptor (RXR)-Selective Agonists: Analogues of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene) and 6-(Ethyl(5,5,8,8-tetrahydronaphthalen-2-yl)amino)nicotinic Acid (NEt-TMN). *J Med Chem.* 2016;59(19):8924-8940.

18. Riancho J, Ruiz-Soto M, Berciano MT, Berciano J, Lafarga M. Neuroprotective effect of bexarotene in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *Front Cell Neurosci*. 2015;9:250.
19. Habchi J, Arosio P, Perni M, et al. An anticancer drug suppresses the primary nucleation reaction that initiates the production of the toxic Abeta42 aggregates linked with Alzheimer's disease. *Sci Adv*. 2016;2(2).
20. Casali BT, Reed-Geaghan EG, Landreth GE. Nuclear receptor agonist-driven modification of inflammation and amyloid pathology enhances and sustains cognitive improvements in a mouse model of Alzheimer's disease. *J Neuroinflammation*. 2018;15:43.
21. Balducci C, Paladini A, Micotti E, et al. The continuing failure of bexarotene in alzheimer's disease mice. *J Alzheimers Dis*. 2015;46(2):471-482.
22. Cummings JL, Zhong K, Kinney JW, et al. Double-blind, placebo-controlled, proof-of-concept trial of bexarotene in moderate Alzheimer's disease. *Alzheimers Res Ther*. 2016;8:2.
23. Santin, E.P.; Germain, P.; Quillard, F.; Khanwalkar, H.; Rodriguez-Barrios, F.; Gronemeyer, H.; de Lera, A.R.; Bourguet, W. Modulating Retinoid X Receptor with a Series of (E)-3-[4-Hydroxy-3-(3-alkoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)phenyl]acrylic Acids and Their 4-Alkoxy Isomers. J. Med. Chem. 2009, 52, 3150-3158.
24. Fujii, S.; Ohsawa, F.; Yamada, S.; Shinozaki, R.; Fukai, R.; Makishima, M.; Enomoto, S.; Tai, A.; Kakuta, H. Modification at the Acidic Domain of RXR Agonists has Little Effect on Permissive RXR-heterodimer Activation. Bioorg. Med. Chem. Lett. 2010, 20(17), 5139-5142.
25. Jurutka, P.W.; Kaneko, I.; Yang, J.; Bhogal, J.S.; Swierski, J.C.; Tabacaru, C.R.; Montano, L.A.; Huynh, C.C.; Jama, R.A.; Mahelona, R.D.; Sarnowski, J.T.; Marcus, L.M.; Quezada, A.; Lemming, B.; Tedesco, M.A.; Fischer, A.J.; Mohamed, S.A.; Ziller, J.W.; Ma, N.; Gray, G.M.;

van der Vaart, A.; Marshall, P.A.; Wagner, C.E. Modeling, Synthesis, and Biological Evaluation of Potential Retinoid X Receptor (RXR) Selective Agonists: Novel Analogues of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene) and (E)-3-(3-(1,2,3,4-tetraydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)-4-hydroxyphenyl)acrylic Acid (CD3254). J. Med. Chem. 2013, 56, 8432-8454.

26. Marshall, P.A.; Jurutka, P.W.; Wagner, C.E.; van der Vaart, A.; Kaneko, I.; Chavez, P.I.; Ma, N.; Bhogal, J.S.; Shahani, P.; Swierski, J.C.; MacNeill, M. Analysis of Differential Secondary Effects of Novel Rexinoids: Select Rexinoid X Receptor Ligands Demonstrate Differentiated Side Effect Profiles" Pharma. Res. Per. 2015, 3(2), e00122, doi: 10.1002/prp2.122.
27. Kakuta, H.; Yakushiji, N.; Shinozaki, R.; Ohsawa, F.; Yamada, S.; Ohta, Y.; Kawata, K.; Nakayama, M.; Hagaya, M.; Fujiwara, C.; Makishima, M.; Uno, S.; Tai, A.; Maehara, A.; Nakayama, M.; Oohashi, T.; Yasui, H.; Yoshikawa, Y. RXR Partial Agonist CBt-PMN Exerts Therapeutic Effects on Type 2 Diabetes without Side Effects of RXR Full Agonists. ACS Med. Chem. Lett. 2012, 3(5), 427-432.
28. Yuan S, Chan JFW, Chik KKH, et al. Discovery of the FDA-approved drugs bexarotene, cetilistat, diiodohydroxyquinoline, and abiraterone as potential COVID-19 treatments with a robust two-tier screening system. Pharmacol Res. 2020;159:104960.