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Metadichol®. A Novel Inverse Agonist of Aryl Hydrocarbon Receptor (AHR) and NRF2 Inhibitor

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Abstract

Metadichol® a novel nano emulsion of lipid alcohols is an Inverse agonist of Vitamin D Receptor (VDR). In this communication, we show that Metadichol is an inverse agonist of the nuclear receptor AHR (Aryl Hydrocarbon receptor) and also an inhibitor of NRF2 (Nuclear factor (erythroid-derived 2)-like 2)) and is a transcription factor that is ubiquitously expressed at low levels in all human organs and regulates a primary cellular defense mechanism, tight regulation to maintain cellular homeostasis. AHR is highly expressed in a broad panel of tumors, AHR is induced by 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) Metadichol® as an inverse agonist against AHR could be potentially useful in the treatment of such diseases. Strong *in vivo* evidence suggests that TCDD can stimulate cross-talk between AHR and Nrf2. The constitutive up regulation of Nrf2 signaling appears to drive the cellular proliferation and resistance to chemotherapy in various cancers. Therefore, pharmacological inhibition of Nrf2 by Metadichol® holds promise as a therapeutic strategy in chemo resistant forms of cancer.

Keywords: Metadichol; Nano lipid; Long chain saturated alcohols; VDR; Inverse agonist; Protean agonist; AHR; Aryl Hydrocarbon Receptor; Inverse agonist; Antagonist; Chemo resistant cancers; Proliferation; Metastasis; Constitutive activity; NRF2

Introduction

Metadichol® is a nano formulation of long chain alcohols that is an inverse agonist of Vitamin D receptor [1]. AHR (Aryl hydrocarbon receptor) like VDR a ligand activated transcription factor. It forms a nuclear hetero dimer with the AHR nuclear trans locator (ARNT) protein to activate gene expression. AHR [2] is known to mediate most of the toxic and carcinogenic effects of a wide variety of environmental contaminants such as dioxin TCDD (2,3,7,8-tetrachlorodibenzo-[p]-dioxin). Many dietary compounds and products of commensal flora activate AHR signaling at physiologically relevant doses and with significant potency [3,4]. This allows targeting AHR to treat inflammatory and autoimmune diseases [5]. It is conceivable therefore that inhibition of AHR activity by antagonists could result in beneficial anti-inflammatory actions. Evidence for such anti-inflammatory effects has recently been identified by use of the AHR antagonist CH-223191 [6] which represses TH17. A high affinity AHR antagonist, Stemregenin 1 (SR1) has been shown to increase the proliferation of human hematopoietic stem cells *in vitro* [7]. AHR provides a molecular pathway through which environmental factors modulate the immune response and, consequently, the development of immune-mediated disorders. Agonists of the AHR receptor play an integral role in T-cell function, promoting a TH2/TH1 switch resulting in a TH1 bias [8]. AHR is usually present in epithelial cells where it is activated by different ligands. Elevated levels of AHR are seen in breast and other cancers where AHR is always activated by depressing anti-tumor immune responses. AHR up regulation allows tumor cells to migrate, invade local tissue and metastasize to other organs. AHR is highly expressed in a wide variety of tumors. Molecules with antagonistic AHR activity could be considered potential candidates for the treatment of such diseases. AHR is widely expressed in Pancreatic [9], Prostate [10], Urinary tract [11] Lung [12] Esophagus [13], Pituitary [14], gliomas [15] For long-term survival of cancers treatments activating the patient's own immune system is an important approach. For a successful treatment, other aspects of cancer progression, like cancer

stem cells, metastatic behavior and decreased tumor cell apoptosis need to be targeted simultaneously. AHR also plays a role in producing "cancer stem cells", that are resistant to chemotherapies and this leads to lethal relapse and metastasis even years after "successful" chemo- or radiotherapy. Inhibitors not only boost anti-tumor immunity, but they also suppress metastasis and the formation of chemo-resistant cancer stem cells. Inhibiting AHR activity holds the promise of a non-toxic therapy that targets crucial tumor progressing processes at the same time. AHR antagonists have therapeutic immunological value [16].

Nrf2

The Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor regulating a variety of genes for antioxidant and detoxification enzymes such as glutathione S-transferase A2 (GSTA2) and NADPH quinone oxido-reductase 1 (NQO1) in response to oxidative and xenobiotic stress [17]. Antioxidants counteract oxidative stress, employing a variety of techniques to diminish ROS levels in the intracellular environment. Free radicals are elevated during cancer and activate signaling pathways of cell proliferation and migration, and can cause DNA damage leading to mutations. Nrf2 signaling pathway consists of three main components: (a) Keap1, (b) Nrf2, and (c) the Antioxidant response element (ARE). These three elements function respectively as the sensor, controller, and responder [18]. Constitutive activation of Nrf2 occurs in many cancers. Aberrant activation of Nrf2 is correlated to tumor progression, chemo resistance, and radiation resistance. NRF2 activations are regarded as beneficial, but recent work suggests otherwise [19]. Increased NRF2 activity from mutations

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in NFE2L2 or KEAP1 is contributing to chemotherapy resistance. Excessive induction of NRF2 genes in cancer cells, and therefore superior protection against cell stress may confer a survival advantage [20].

NRF2 is being researched as a drug target in many diseases like COPD and Multiple sclerosis and in enhancing cancer cell susceptibility to chemotherapy. Sulforaphane is useful in COPD, rescuing impaired phagocytosis in alveolar macrophages and breaking down macrophage resistance to glucocorticosteroid treatment via HDAC2 denitrosylation [21,22]. Dimethyl fumarate has shown promise in clinical multiple sclerosis trials, in a mouse MS model (experimental autoimmune encephalomyelitis, or EAE). It attenuated some of the damaging effects of the disease in dependence on Nrf2. Treatment enhanced Nrf2 stabilization and activity [23]. Ren et al. [24] studied the effect of Brusatol a compound found in a plant extract that inhibits the NRF2 pathway. On lung cancer cells Brusatol increases ubiquitination of NRF2 leaving other signaling pathways unaffected, and renders both cancer cell lines and xenografts susceptible to the chemotherapeutic agents like Cisplatin.

Nrf2 is essential to maintaining the cellular redox homeostasis, providing protection against oxidative or electrophilic stresses, and suppressing tumor promotion and progression [25]. *In vivo*, Nrf2-knockout mice show greater damage in organs such as the lung, liver, and kidney when exposed to chemical toxins such as cigarette smoke or hypoxia [26]. Additionally, Nrf2-knockout mice are more prone to developing diseases related to oxidative stress and cancers [27]. Constitutive elevation of Nrf2 expression and Nrf2-ARE activity occurs in many cancers and is involved in their chemo-resistance and radiation resistance. Ideally, inhibitors should block the Nrf2-ARE signaling pathway in cancer cells, while leaving healthy cells unaffected. Although expression of Nrf2 can be suppressed experimentally by gene therapy, implementing them clinically in cancer treatment is a major hurdle. It is important to identify small-molecular Nrf2 inhibitors that block Nrf2 signaling pathways specifically in cancer cells. Thus, there is a need for a molecule that is specific and has bioactivity and also has no toxicity. Metadichol® as we shall show may fit that need.

NRF2 and AHR

Both AHR and NRF2 signaling regulate the expression of genes affecting the metabolism of xenobiotics. Miao et al. [28] showed that Nrf2 gene transcription is modulated by AHR activation. NRF2 also directly modulates AHR signaling, highlighting the dependent nature of interactions between these two pathways [29]. NRF2 controls AHR signaling to expand its function of NRF2 to include the metabolism to include xenobiotics and carcinogens via CYPs and adipogenesis. AHR and Nrf2 double knockout mice studies offer additional evidence for interactions between these pathways [30]. Cooperation between AHR and Nrf2 has biological implications. AHR has to do with carcinogenesis, whereas Nrf2 is involved with cytoprotection. The challenge is to exploit this to a therapeutic advantage and to improve chemo preventive strategies.

Experimental

AHR nuclear receptor assay was outsourced and performed by Indigibio sciences PA, USA.

Reporter cells were treated with seven concentrations of respective test compounds, starting at 10 mg/ml and following with six more 3.33-fold dilutions. All treatment concentrations were performed in triplicate. Reporter cells were also treated with vehicle only as a

control. Performance of the human NR agonist and antagonist assays were validated by performing a Reference Agonist (MeBIOp), and Antagonist (GNF 351) dose response curves

Assay methods

Plasmids. This study utilized proprietary nuclear receptor reporter cells expressing either the full-length nuclear receptor sequence (AHR,) or a hybrid receptor comprising the N-terminal Gal4 DNA binding domain fused to the ligand binding domain of the specific human nuclear receptor. The reporter vectors used in these studies comprise the firefly luciferase gene functionally linked to either an upstream NR response element (NRE) or the Gal4 activation sequence (UAS).

Compound handling

Test compound was delivered as solution dissolved in water. Stock of test compounds was stored at room temperature as directed by the sponsor. Setup of NR Assays. The NR Assays were performed as depicted in Figures 1 and 2. In brief, Step 1: A suspension of Reporter cells was prepared in cell recovery medium (CRM; containing 10% charcoal stripped FBS). For antagonist assays, reporter cells were supplemented with a 2x-EC80 concentration of the appropriate reference agonist. For agonist and antagonist assays, 100 ml of the reporter cell suspension was dispensed into the wells of white, cell culture treated, 96-well assay plates. For agonist and antagonist assays, 2x-concentration treatment media were prepared.

Step 2: Immediately prior to assay setup, test compounds were diluted using compound screening medium (CSM; containing 10% charcoal stripped FBS) to generate '2x-concentration' treatment media. 100 ml of each treatment medium was dispensed into triplicate assay wells pre-dispensed with Reporter Cells. Assay plates were incubated at 37°C for 24 hr.

Step 3: Following the 24 hr incubation period, treatment media were discarded and 100 µl/well of Luciferase Detection Reagent was added. RLU's were quantified from each assay well to determine NR activity.

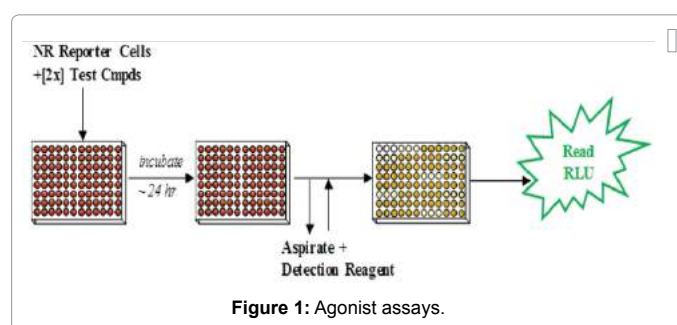


Figure 1: Agonist assays.

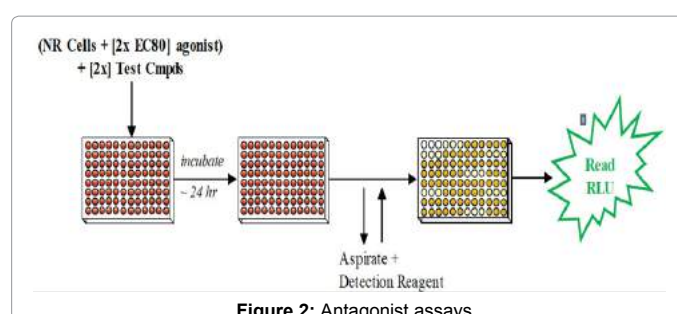


Figure 2: Antagonist assays.

Assay validation

Reference compounds were utilized to confirm the performance of the specific lot of NR Reporter Cells treated with the Sponsor's test compounds. Reference compound and test compound assays were performed at the same time and, hence, were exposed to the same assay reagents and environmental conditions. Refer to individual data sets for the identities of specific reference agonist and antagonist and their respective treatment concentration ranges. Reference groups always include a 'Vehicle' control to determine background activity in the assay and to calculate fold-activation or percent-inhibition.

Data reduction

Microsoft Excel was used to manage and archive assay data, as well as to calculate average RLU values +/- Standard Deviation (SD), Fold-activation, Percent activation, Percent Coefficients of Variation (%CV), and Z' values.

Graphical data methods

Dose-response curve (DRC) analyses of the reference compounds and test compounds were performed via non-linear curve-fitting of Fold-Activation vs. Log(Compound) for agonist assays and percent inhibition vs. log(Compound) for antagonist and inverse agonist assays, using Graph Pad Prism software.

Keap1-NRF2 assays

All work was outsourced and performed by DiscoverX, Fremont, CA, using their proprietary procedure. This is a target-specific, cell-based functional assay for NRF2 that provides a non-transcriptional response of activation-dependent NRF2 translocation to the nucleus.

Assay measures

Nuclear translocation of NRF2 following its disassociation from Keap1

Activation of the NRF2 response pathway

The control ligand was CDDO methyl ester.

Experimental Procedure

Compounds were tested in agonist and antagonist mode with the Keap1-NRF2 Biosensor Assay. For agonist assays, data was normalized to the maximal and minimal response observed in the presence of control ligand and vehicle. For antagonist assays, data was normalized to the maximal and minimal response observed in the presence of EC80 ligand and vehicle. An EC80 of 12 nM CDDO methyl ester was used.

Cell handling

1. PathHunter Pathway cell lines were expanded from freezer stocks according to standard procedures.

2. Cells were seeded in a total volume of 20 μ L into white walled, 384-well micro plates and incubated for the appropriate time prior to testing.

Agonist format

1. For agonist determination, cells were incubated with sample to induce response.

2. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer.

3. 5 μ L of 5X sample was added to cells and incubated at 37°C or

room temperature for 1.5-16 hours depending on the assay. Vehicle concentration was 1%.

Antagonist format

1. For antagonist determination, cells were pre-incubated with antagonist followed by agonist challenge at the EC80 concentration.

2. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer.

3. 5 μ L of 5X sample was added to cells and incubated at 37°C or room temperature for 60 minutes. Vehicle concentration was 1%.

4. 5 μ L of 6X EC80 agonist in assay buffer was added to the cells and incubated at 37°C or room temperature for 1.5-16 hours depending on the assay.

Signal detection

1. Assay signal was generated through a single addition of 12.5 or 15 μ L (50% v/v) of PathHunter Detection reagent cocktail for agonist and antagonist assays respectively, followed by a one-hour incubation at room temperature. For some assays, activity was detected using a high sensitivity reagent (PathHunter FlashKit) to improve assay performance. In these assays, equal volume of detection reagent (25 or 30 μ L) was added to the wells, followed by a one hour at room temperature.

2. Micro plates were read following signal generation with a Perkin-Elmer EnvisionTM instrument for chemiluminescent signal detection

Data analysis

1. Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA).

2. For agonist mode assays, percentage activity was calculated using the following formula: % Activity = 100% x (mean RLU of test sample - mean RLU of vehicle control) / (mean MAX RLU control ligand - mean RLU of vehicle control).

3. For antagonist mode assays, percentage inhibition was calculated using the following formula:

% Inhibition = 100% x (1 - (mean RLU of test sample - mean RLU of vehicle control) / (mean RLU of EC80 control - mean RLU of vehicle control)).

Results and Discussion

The AHR raw data results for agonist and antagonist are shown in Tables 1 and 2, The agonist and antagonist graphs are shown in Figures 3-8. MeBio was used as a standard in the agonist assay and GNF-351 used as the standard in the antagonist assay. Keap1-NRF2 raw data are shown in Tables 3-5. From the Nuclear receptor agonist and antagonist assay Figures 5 and 6 it is clear that Metadichol is behaving like an inverse agonist. There is no known inverse agonist of AHR reported in literature.

In the Keap-NRF2 assay shown in Figure 7 it clear that Metadichol has no agonist activity but has an antagonist activity. But what is interesting is that the response is not 100% even at higher concentration and maximum response did not exceed 35%.

Nutrition has a huge role in functioning of the human organism by affecting gene expression in specific tissues of the organism. This helps the organism to adapt to changes in the environment. The intracellular Nuclear receptors link diet and gene expression in response to small

Compound	Values									
Water	0.20%	15,067	11,180	10,349	12,199	2,518	1.0	4.2	21	-
Metadichol (µg/ml)	0.0073	10,986	8,085	10,559	9,877	1,566	0.81	3.4	16	-
	0.024	9,304	7,012	5,776	7,364	1,790	0.60	2.5	24	-
	0.081	8,291	6,874	7,178	7,448	746	0.61	2.5	10	-
	0.27	7,445	7,537	6,870	7,284	361	0.60	2.5	5.0	-
	0.90	3,513	5,732	5,247	4,830	1,167	0.40	1.6	24	-
	3.0	3,056	2,112	2,966	2,712	521	0.22	0.92	19	-
	10	302	262	286	283	20	0.023	0.10	7.1	-
Reference Antagonist: GNF351 (nM)	0.0038	84,571	80,274	96,520	87,121	8,418	7.1	30	9.7	Z'
	0.19	110628	130,696	92,272	111,199	19,218	9.1	38	17	-
	0.96	184984	156,008	155,234	165,409	16,957	14	56	10	-
	4.8	229832	217,538	173,853	207,074	29,420	17	70	14	0.51
	24	282781	262,276	269,296	271,451	10,421	22	92	3.8	0.85
	120	238531	300,398	297,017	278,649	34,784	23	95	12	0.58
	600	284983	326,358	270,364	293,902	29,043	24	100	10	0.66
3,000	266265	347,989	235,103	249,786	15,658	20	85	6.3	0.77	

Table 1: Agonist assay.

Compound	Conc.	Human AhR antagonist assays			AVG	SD	Fold-inhibition	% Inhibition	% CV	Value
		Luc1	Luc2	Luc3						
Water	0.20%	231,269	274,275	272,890	259,478	24,440	1.0	0.0	9.4	-
Metadichol (µg/ml)	0.0073	283,234	226,097	297,118	268,860	37,667	0.97	-3.6	14	-
	0.024	266,507	285,157	280,584	277,416	9,720	0.94	-6.9	3.5	-
	0.081	266,802	263,538	285,246	271,862	11,705	0.95	-4.8	4.3	-
	0.27	231,747	225,736	243,391	233,625	8,976	1.1	10	3.8	-
	0.90	140,583	147,609	127,846	138,679	10,018	1.9	47	7.2	-
	3.0	47,538	39,305	26,603	37,815	10,547	6.9	85	28	-
	10	1,216	766	1,264	1,082	275	240	100	25	-
Reference Antagonist: GNF351 (nM)	0.61	267,661	254,185	214,007	245,284	27,912	1.1	5.5	11	Z'
	2.4	235,944	271,376	260,974	256,098	18,212	1.0	1.3	7.1	-
	9.8	269,755	247,412	204,923	240,697	32,934	1.1	7.2	14	-
	39	241,635	231,420	226,931	233,329	7,536	1.1	10	3.2	-
	156	175,211	154,885	188,760	172,952	17,050	1.5	33	10	-
	625	67,127	70,861	94,960	77,649	15,107	3.3	70	19	-
	2500	10,709	11,052	14,977	12,246	2,371	21	95	19	0.67
10000	202	128	218	183	48	1420	100	26	0.72	

Table 2: Antagonist assay.

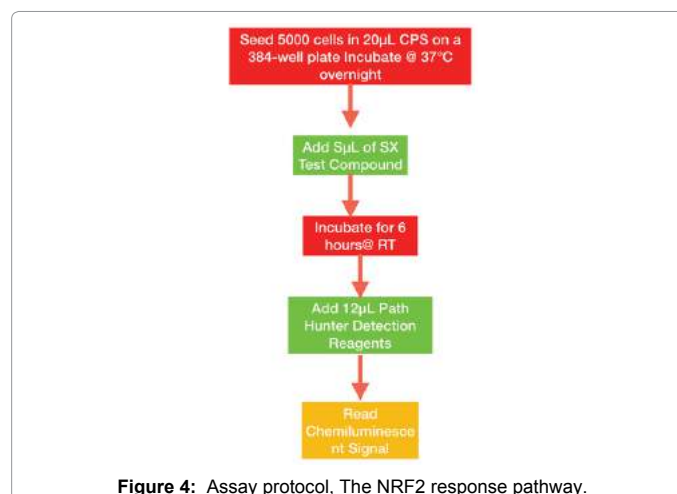
- **Percent Coefficient of Variation (%CV)** is: $100 * (SD / Ave. RLU)$
- **Fold-Activation** in Agonist assays is: $[Ave RLU_{Test Cmpd} / Ave RLU_{Vehicle}]$
- **Percent-Activation of Ref^{MAX}** is calculated by normalizing test cmpd RLU values to the maximum RLU value of the reference agonist (=100%), as follows: $100 * (Ave. RLU_{Test Cmpd} / Ave RLU_{Reference Maximum})$
- **Z' for Reference Agonists** is: $1 - [(3 * (SD_{Vehicle} + SD_{Ref Cmpd})) / (RLU_{Ref Cmpd} - RLU_{Vehicle})]$
- **Fold-Inhibition in Antagonist** is: $[Ave RLU_{EC50 agonist} / Ave RLU_{Test Cmpd}]$
- **Percent-Inhibition in Antagonist.** The theoretical minimum inhibition (0% inhibition) derives from EC₅₀ agonist treatment only, no treatment cmpd. % Inhibition is calculated as: $100 * (1 - [Ave RLU_{Test Cmpd} / Ave RLU_{EC50 agonist}])$

Figure 3: Microsoft Excel was used to manage and archive assay data.

lipophilic ligands derived from endocrine organs, metabolism, diet and the environment. Metadichol is composed of C-26, C-28, C-30 long chain alcohols and is an open chain analogue of 1,25 dihydroxy vitamin D3 the natural ligand of VDR which is a C27 alcohol. In addition, Metadichol is ubiquitous lipid that is part of our diet [31] and is a lipophilic ligand. Most of the ligands for the known

Nuclear receptors are lipids with the exception of the Thyroid receptor which has Iodine present. [32].

An inverse agonist binds to the same binding site on the receptor



as an agonist does but induces a biological response opposite to that of an agonist. A prerequisite for an inverse agonist response is that the receptor must have high constitutive or basal activity [33] in the absence of an identifiable ligand. An inverse agonist [34] can have effects

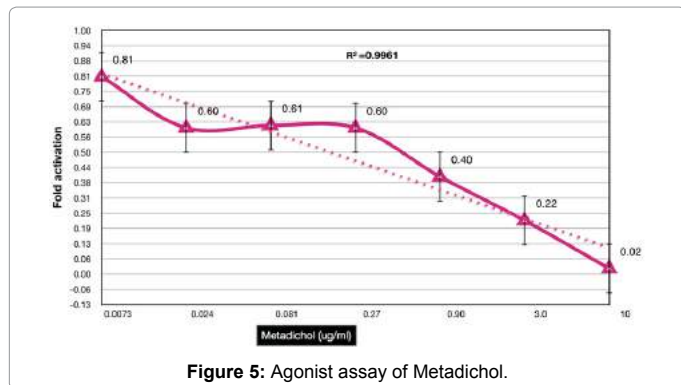


Figure 5: Agonist assay of Metadichol.

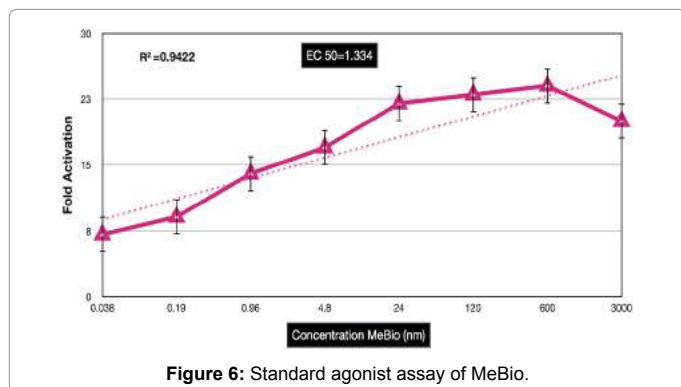


Figure 6: Standard agonist assay of MeBio.

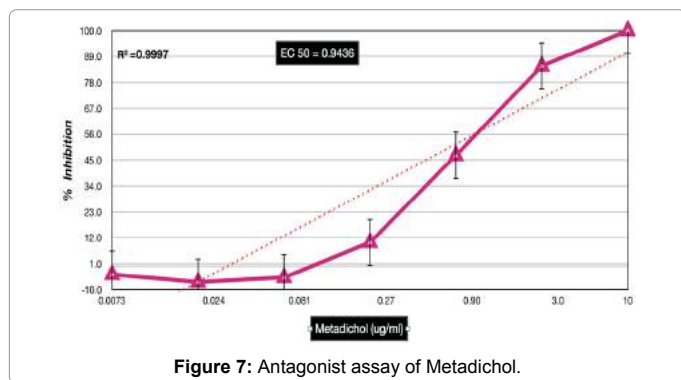


Figure 7: Antagonist assay of Metadichol.

CDDO Methyl Ester			
Well ID	Conc	Raw Value	Percent Efficacy
B22	5.08E-06	237800	2.0
B19	1.52E-05	232800	1.4
B20	1.52E-05	244000	2.8
B17	4.57E-05	221200	0.1
B18	4.57E-05	219000	-0.2
B15	0.00013717	226400	0.7
B16	0.00013717	241400	2.5
B13	0.00041152	253400	3.9
B14	0.00041152	262800	5.0
B11	0.0012346	367200	17.2
B12	0.0012346	352200	15.5

B9	0.0037037	685600	54.6
B10	0.0037037	624400	47.4
B7	0.011111	927200	82.9
B8	0.011111	942400	84.7
B5	0.033333	1E+06	94.2
B6	0.033333	1E+06	105.8
B3	0.1	1E+06	96.0
B4	0.1	1E+06	110.4

Table 3: Keap1-NRF2 raw data.

Metadichol-Agonist assay	Conc	Raw value	Percent efficacy
C21	9.77E-02	273400	1.3
C22	9.77E-02	275400	1.5
C19	1.95E-01	254200	-1.1
C20	1.95E-01	281200	2.3
Cu7	3.91 E-01	257000	-0.7
C18	3.91 E-01	248800	-1.7
C15	0.78125	256200	-0.8
C16	0.78125	267800	0.6
C13	1.5625	246800	-2.0
C14	1.5625	244400	-2.3
C11	3.125	255400	-0.9
C12	3.125	255200	-1.0
C9	6.25	268600	0.7
C10	6.25	279200	2.0
C7	12.5	296000	4.1
C8	12.5	277000	1.7
C5	25	269600	0.8
C6	25	266000	0.4
C3	50	238000	-3.1
C4	50	257000	-0.7

Table 4: Metadichol-agonist assay Keap1-NRF2 raw data.

Metadichol antagonist assay	Conc	Raw val	Percent efficacy
H21	9.77E-02	1195800	-3.5
H22	9.77E-02	1252000	-9.8
H19	1.95E-01	1239600	-8.4
H20	1.95E-01	1124000	4.5
H17	3.91 E-01	1125000	4.4
H18	3.91 E-01	1125400	4.3
H16	0.78125	1050000	12.8
H16	0.78125	1116800	5.3
H13	1.5625	1001000	18.2
H14	1.5625	1072800	10.2
H11	3.125	977800	20.8
H12	3.125	913000	28.1
H9	6.25	984400	20.1
H10	6.25	986800	19.8
H7	12.5	871000	32.8
H8	12.5	914600	27.9
H5	25	886000	31.1
H6	25	879600	31.8
H3	50	853800	34.7
H4	50	831400	37.2

Table 5: Metadichol-antagonist assay Keap1-NRF2 raw data.

similar to that of an antagonist but cause a distinct set of downstream biological responses. They block the effects of binding agonists like a classical antagonist but also inhibit the basal activity of the receptor.

Antihistamines were classified previously as antagonists of histamine H1 receptors have been reclassified as inverse agonists [35]. AHR is constitutively active in prostate cancer [36,37]. Tumors and tumor cell lines have elevated AHR levels. AHR is chronically activated in tumors [38]. Masafumi et al. [39] created a transgenic mouse lines expressing the constitutive form of the AHR in keratinocytes. In these lines of mice, and showed that the AHR activity was constitutively enhanced in the absence of ligands, and direct effects of PAHs (poly aromatic hydrocarbons) and their metabolites could be ignored.

The enhanced level of expression of AHR in human tumors is the ability of NFkB to increase AHR expression, which correlates with inflammatory status [40]. There is a need for specific high-affinity AHR

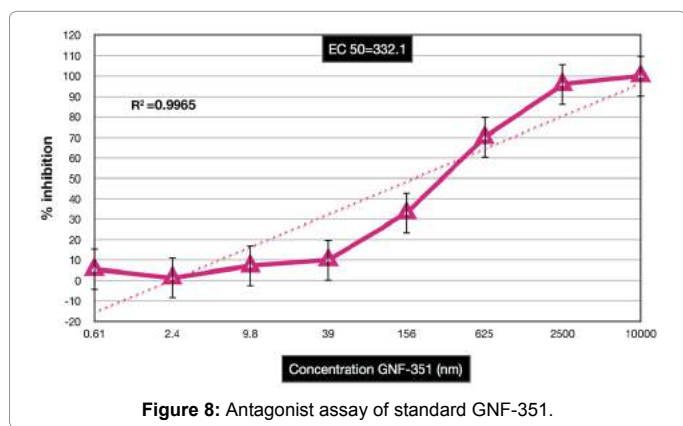


Figure 8: Antagonist assay of standard GNF-351.

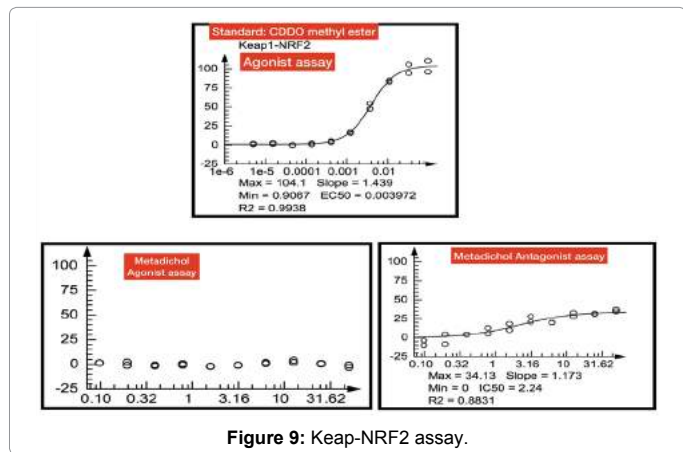


Figure 9: Keap-NRF2 assay.

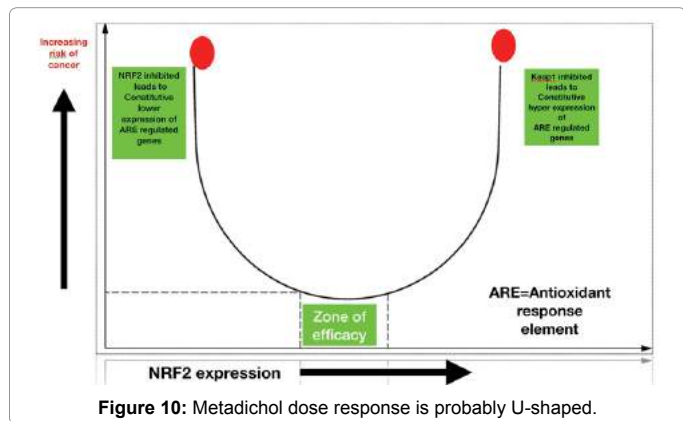


Figure 10: Metadichol dose response is probably U-shaped.

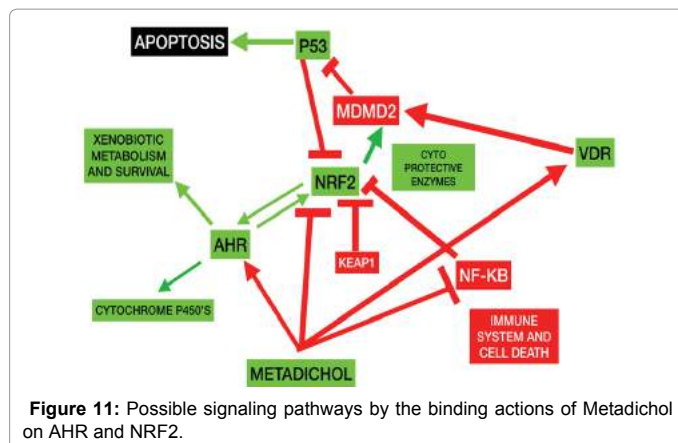


Figure 11: Possible signaling pathways by the binding actions of Metadichol on AHR and NRF2.

ligands that exhibit improved biological profiles. Metadichol, as we have shown, is an inhibitor of NF-KB. The emerging role of the AHR in immune tolerance, and clinical testing of AHR antagonists like Metadichol as an adjunct to immunotherapy is an approach that needs to be pursued. We have already shown that Metadichol is an inverse agonist of VDR. No known inverse agonist of AHR and VDR are known. What has been postulated is that an inverse agonist will have effects opposite to that of an agonist. Inverse agonists not only block constitutive responses of receptors but also to activate and regulate seven-transmembrane receptor (GPCR) signaling [41]. Our studies in human subjects suggest that Metadichol behaves more like a protean agonist [42,43]. For example, we have shown that it lowers insulin levels as well as increases Insulin levels. We believe that it also acts as a protean agonist i.e. it acts as an inverse agonist where there is constitutive activity and as an agonist when there is none.

NRF2 and Metadichol

In the Keap-NRF2 assay shown in Figure 9 it clear that Metadichol has no agonist activity but has an antagonist activity. But what is interesting is that the response is not 100% even at higher concentration, and maximum response does not exceed 35%. The barrier to be overcome in cancer treatment is the resistance of cancer cells to chemotherapy. High constitutive expression of Nrf2 is found in many types of cancers, that leads to an environment conducive to cancer cell survival. Thus, there is a need for optimal inhibition of NRF2 expression to overcome the resistance of cancer cells to chemotherapy. Metadichol dose response is probably U-shaped (Figure 10). U-shaped curves are seen with some chemo preventive agents, such as vitamin D and selenium as well as for some chemotherapeutic drugs. Metadichol does not activate NRF2 and thus could be useful to increase the effectiveness of cancer treatment. [44]. The nrf2 pathway is the useful target in the treatment of diabetes [45]. We have published a case study where Metadichol normalized blood sugar levels [46].

Conclusion

The results that we have obtained with Metadichol shows that it is an inverse agonist of AHR. Possible signaling pathways by the binding actions of Metadichol on AHR and NRF2 is shown in Figure 11. Thus, Metadichol could be useful in cancers where there are increased levels of AHR expressed. The discovery of functional cooperation between AHR and Nrf2 has significant biological implications [47,48]. AHR has been associated with carcinogenesis, but Nrf2 is associated with

cytoprotection against degenerative diseases. Cross-talk between these two transcription factors can be exploited by Metadichol to therapeutic advantage and improve outcomes in cancer chemotherapy where there is a need to control AHR and NRF2 level. Metadichol by binding to VDR could be useful given the role of Vitamin D in cancer therapy [49]. Metadichol by leveraging targets AHR, VDR, NRF2 that are synergistic can produce greater levels of efficacy and synergy combinations and positively impacts the complex systems biology of human disease. Being not toxic [50-52] and is a huge advantage over all drugs in development and allows for clinical testing in humans.

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