Metadichol® A Novel Inverse Agonist of Thyroid Receptor and its Applications in Thyroid Diseases

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Metadichol® A Novel Inverse Agonist of Thyroid Receptor and its Applications in Thyroid Diseases

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Abstract
An estimated 200 million individuals worldwide have a thyroid disorder. Thyroid diseases affect seven times more women than men. People not diagnosed make up the majority of thyroid patients. There is a need to find novel and safe ways to change the underlying disease processes, rather than merely stop excess thyroid hormone production as in hyperthyroidism.

Metadichol® is a nano emulsion of an extract of long-chain alcohols from food that is an inverse agonist of VDR (Vitamin D receptor), AHR (Aryl Hydrocarbon Receptor), and RORC (RAR Related Orphan Receptor C). The work presented here shows that Metadichol® is an inverse agonist of THRA (Thyroid Receptor Alpha) and THR5 (Thyroid Receptor Beta). Case studies are presented that show how it can safely treat a multitude of thyroid related diseases. Network and pathway enrichment studies are presented that show how Metadichol® may be involved in action on multiple receptors and exerting its effects through multiple pathways. Metadichol® is the first of a breed of molecules that moves the goal post from the concept of ‘one drug, one target’ toward simultaneously targeting multiple targets, that can potentially lead to successful treatment of many diseases. Given the safety profile of Metadichol®, it would not only mitigate thyroid disease but prevent it and reducing the burden on healthcare budgets worldwide.

Keywords
(Vitamin D receptor) VDR; Metadichol; Nano emulsion; Thyroid Nuclear receptors; THRA (Thyroid Receptor Alpha); THR5 (Thyroid Receptor Beta); Inverse and Protein agonists; Hypothyroidism; Hyperthyroidism; Thyroid antibodies; Hashimoto; Graves’ disease

Introduction
Thyroid disease affects about 12 percent of the US population. While many people with thyroid disease don’t even know they have it, an overactive or underactive thyroid can cause a slew of problems, including weight gain or loss, mood changes and infertility. In children, an underactive thyroid can be fatal, which is why they are tested for a deficiency at birth [1,2].

On a global scale, a staggering 200 million people have problems with their thyroid glands, with over 50 percent remaining undiagnosed. It affects women more disproportionately than men. Thyroid hormones are essential for growth, neuronal development, reproduction and regulation of energy metabolism. Hypothyroidism and hyperthyroidism are common conditions with potentially devastating health consequences that affect all populations worldwide.

The thyroid hormone receptors, THRA and THR5 are members of the nuclear receptor family [3]. The body relies heavily on the thyroid hormone for digestion, the growth of hair and nails, sex drive, and metabolism are dependent on the right levels of thyroid hormone for proper functioning. The thyroid uptakes iodine from diet and converts it into thyroid hormones.

The two main types of thyroid hormones are called L-triiodothyronine (T3) and L-thyroxine (T4). These hormones regulate and control how cells, use oxygen and energy. The synthesis and secretion of thyroid hormones are controlled by feedback loops that involve the hypothalamus, pituitary, and thyroid glands.

The hypothalamus secretes Thyrotropin-Releasing Hormone (TRH), that signals the pituitary that releases Thyroid-Stimulating Hormone (TSH), signaling the thyroid gland to synthesize thyroid hormones. High thyroid hormone levels will inhibit TRH production, decrease TSH secretion from the pituitary, and decrease thyroid hormone synthesis [4].

Thyroid diseases and treatment
Today four drugs are currently being used for treating thyroid diseases: 3,5-diiodothyroproionic acid, amiodarone, L-triiodothyronine, Levothyroxine.

Hypothyroidism is a disorder with multiple causes in which the thyroid fails to secrete an adequate amount of thyroid hormone, this is the most common thyroid disorder usually caused by primary thyroid gland failure and may result from diminished stimulation of the thyroid gland by TSH. Hypothyroidism can be treated with thyroid hormone replacement drugs. Levothyroxine is a stereoisomer of thyroxine (T4) which is degraded much slowly and can be administered once daily in patients with hypothyroidism [5]. Hyperthyroidism results in excess synthesis and secretion of thyroid hormones by the thyroid gland. The synthetic form of the T3 hormone, known as liothyronine is sometimes added to levothyroxine for T4/T3 combination treatment (Table 1).

Hashimoto’s is an autoimmune disease, meaning that the immune system inappropriately attacks your thyroid gland. Thyroid-attacking antibodies produced are Thyroid Peroxidase antibodies (TPO) and Thyroglobulin Antibodies (TgAb). Hashimoto disease leads to conditions that cause inflammation and gradual destruction of thyroid gland over time leading to hypothyroidism.

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Thyroid and vitamin D

Research to date suggest the association of vitamin D deficiency with higher incidence of autoimmune thyroiditis [16,17]. More severe deficiency is often accompanied by thyroid hypofunction [18]. The levels of 25(OH)D3 seem to be an independent factor influencing the presence of TPOAb positivity [19,20].

It has been vitamin D below 12.5 ng/mL is a risk factor for development of thyroid diseases. Whether supplemented with high doses of vitamin D is preventative or could have therapeutic effect is aa (delete aa) still being debate [21]. It has been shown by molecular modelling studies that 1,25-D has a very high affinity [22] for the alpha Thyroid Nuclear Receptor (THRA). Since Metadichol® is an inverse agonist of VDR (Vitamin D Receptor), a thyroid nuclear receptor assay was carried out. It showed that Metadichol is an interestingly an inverse agonist of THRA and THRB. Metadichol is also inhibitor of TNF alpha, MCP1 (CCL2) and ICAM1 [23,24] have a role in thyroid diseases and presented here are case studies that confirm Metadichol® and its potential use in mitigating Thyroid diseases.

Methods

Experimental nuclear receptor assay

Nuclear receptor (NR) assays: The assays were performed by Indigo Biosciences Inc, PA, USA using their proprietary assay methods.

Plasmids: This study utilized a hybrid receptor comprising the N-terminal Gal4 DNA binding domain fused to the ligand binding domain of the specific human nuclear receptor (THRA and THRB). 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.

Set up of NR Assays: The NR Assays were performed as depicted in Figures 1 and 2.

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 hours.

| Hypothyroidism | TSH | T4 | T3 |
|---------------|-----|----|----|
| Hyperthyroidism | low | high | high |

Table 1: Typical Thyroid Hormone Levels in Thyroid Disease.

Graves’ disease is an autoimmune disorder that causes hyperthyroidism, or overactive thyroid. This is caused by overstimulation of the thyroid. Graves’ disease leads to goiter and in some cases thyroid nodules. This leads to cancer of the thyroids that is most often found in nodules in the thyroid gland. Hyperthyroidism caused by Graves’ disease may be treated with the thioamide drugs propylthiouracil, carbimazole or methimazole, and rarely with Lugol’s solution. Additionally, hyperthyroidism and thyroid tumors may be treated with radioactive iodine.

Thyroid cancer almost always involves removal of the thyroid gland surgically or need Radioactive Iodine treatment (RAI). Patients after surgery are hypothyroid and require lifelong thyroid hormone replacement treatment. In some cases, thyroid diseases and conditions can have no symptoms at all, such as thyroid cancer or certain types of thyroiditis.

Thyroid diagnosis

Diagnosis of a thyroid condition is usually by blood tests or imaging tests. Blood testing: Levels of (TSH) test, free thyroxine (Free T4) and free triiodothyronine (Free T3) and TPO, TgAB testing and for reverse T3 levels. Imaging tests include CT, MRI and ultrasound scans were carried out to further evaluate the size, shape, and function of the thyroid gland.

There is today a paucity of drugs for treating thyroid diseases and a need for clinically safe molecule. Thyroid antagonists in general have dual THRA and THRB activities. Development of selective THRA and THRB small molecule antagonists has not been considered clinically relevant. A specific small molecule thyroid antagonist is, therefore, a desirable goal of the pharmaceutical industry to treat hyperthyroidism. An increase in circulating thyroid agonists will lead to hyperthyroidism. Whereas a loss in thyroid receptor gene signaling leads to hypothyroidism. Compounds exhibiting thyroid antagonism can cause serious side effects. Drugs amiodarone have thyroid antagonist properties and can induce hypothyroidism in some people.

Thyroid and role of cytokines

A thyroid disease is an inflammatory state characterized by elevated cytokines. Modulating cytokine responses hold considerable promise in the treatment of thyroid diseases [6]. Xinyang et al. showed that constitutively activated NF-kB pathway also closely links Hashimoto’s thyroiditis with increased incidence of thyroid cancers and inhibition of NF-kB, showed alone or with other signaling pathway inhibitors may be of significant therapeutic benefits against aggressive thyroid cancers [7]. Diez et al. reported that high plasma concentrations of TNF-alpha and sTNFR-I are seen in patients with hypothyroidism or hyperthyroidism [8]. Fukazawa et al. showed Intercellular Adhesion Molecule 1 (ICAM1) in the sera of patients with Grave’s Disease [9]. This feature may be an important factor in the progression of cancer of the thyroid gland. Similar work was reported by Zhang et al. that ICAM1 expression is upregulated in PTC (Papillary Thyroid Cancer) and in HT (Hashimoto’s disease) [10]. Buitrago et al. showed that ICAM1 was upregulated in PTC at the levels of both gene and protein expression. Moreover, this upregulated expression correlated with aggressive tumor features [11].

Kemp et al. [12] confirmed expression of CCL2 (MCP-1), levels were associated with serum levels of antithyroid peroxidase (anti-TPO Ab) and antithyroglobulin antibodies (anti-TG Ab). This was confirmed by Wahl et al. [13]. Kokkotou et al. [14] showed that serum CCL2 is increased in women with chronic autoimmune thyroiditis, with family history of hyperthyroidism, suggesting a pathogenetic role for CCL2 in this condition as confirmed by another study by Didushko et al. [15].
Step 3: Following the 24 hour incubation period, treatment media were discarded and 100 all/well of Luciferase Detection Reagent was added. RLUs were quantified from each assay well to determine NR activity.

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 (Table 2).

Dose Response Curve (DRC) analyses of the reference compounds and test compounds were performed via non-linear curve-fitting of Fold-activation vs. compound for agonist assays. Percent inhibition vs. compound for antagonist and inverse agonist assays. Graph Pad Prism software was used for calculations (Tables 3-6) (Figures 3-8).

Results of Experimental Assays

Results of case studies

Patient No 1: Male-38 (figures 9-14)

38 year old male with episodes of syncope associated with seizure activity. It all began with a routine Hepatitis B injection and soon after he began getting seizures. Neurological tests were normal. Was initially reated with anticonvulsants Dilantin and Keppra but the seizure episodes have continued? In addition he suffered from double vision, auras and episodes of dizziness. Most episodes occurred during night while sleeping. He usually collapses, and is completely unconscious for a period of time (15-30 minutes) during which time he may or may not have any movements. He returns to full rational consciousness after the end of these seizures. Needed to be hospitalized after such episodes he used to feel tired and rests for 1-2 days before he feels normal. He was finally diagnosed with Hashimoto encephalopathy with elevated levels of Thyroid antibodies. Treated with Metadichol @ dosage of 10 mg per day in addition to other medications and began to show improvements.

Though s eizures c ontinued t he f requency l essened a nd w ith Metadichol uses so far only mild seizure for 30 seconds and patient returned to normal after 10 minutes as opposed to 1-2 days before and without need to be hospitalized. This improvement was reflected in his biomarkers levels. Patient reports normal blood pressure. Frequent seizures have decreased and since starting with Metadichol. Patient has stopped use of all medications. Patient reports he has very energetic and feels less stressed mentally about his condition.

Patient No 2: Male-83 (figure 15)

Male-83, lung cancer patient. Metadichol @ 10 mg per day. Patient was on chemotherapy and not on any thyroid medications. TSH levels returned to normal range.

Patient No 3: Female-74 (figure 16)

74 years old female with hypothyroid and co morbidities of cardiovascular diseases, anemic and with low energy levels not on any Thyroid medication. Patient reports improved energy levels after use of Metadichol @ 5 mg per day.
• 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 )

• Fold-Inhibition in Antagonist is:
[EC80 agonist / Ave RLU Test Cmpd ]

• Percent-Inhibition in Antagonist:
The theoretical minimum inhibition (0% inhibition) derives from EC80 agonist treatment only, no treatment cmpd. % Inhibition is calculated as:
100*( 1-[Ave RLU Test Cmpd  / Ave RLU EC80 agonist ])

Table 2: Graphical data methods.

| Compound | Conc. | Human TRa Agonist Assays, Host cell line: HEK |
|----------|-------|-----------------------------------------------|
|          |       | Luc1  | Luc2  | Luc3  | AVG  | SD  | Fold-Activation | %Activation of RefMax | % CV |
| Water    | 0.20% | 386   | 254   | 318   | 319  | 66  | 1              | 0.025               | 21   |
| Metadichol (mg/mL) | 0.0073 | 342   | 326   | 428   | 365  | 55  | 1.1             | 0.029               | 15   |
|          | 0.024 | 328   | 270   | 302   | 300  | 29  | 0.94            | 0.024               | 9.7   |
|          | 0.081 | 288   | 306   | 354   | 316  | 34  | 0.99            | 0.025               | 11   |
|          | 0.27  | 254   | 280   | 282   | 272  | 16  | 0.85            | 0.021               | 5.7   |
|          | 0.9   | 288   | 266   | 228   | 261  | 30  | 0.82            | 0.021               | 12   |
|          | 3     | 286   | 206   | 204   | 232  | 47  | 0.73            | 0.018               | 20   |
|          | 10    | 224   | 144   | 156   | 175  | 43  | 0.55            | 0.014               | 25    |
| Reference Agonist: Triidothyronine (nM) | 0.029 | 526   | 454   | 394   | 458  | 66  | 1.4             | 0.036               | 14.7   |
|          | 0.12  | 3,335 | 2,734 | 2,186 | 2,752 | 574 | 8.6             | 0.22               | 21    |
|          | 0.47  | 72,967| 64,891| 61,845| 66,568| 5,748| 208          | 5.3               | 0.74  |
|          | 1.9   | 6,00,780| 3,91,861| 3,67,490| 4,53,377| 1,28,235| 1,420    | 36          | 0.15  |
|          | 7.5   | 10,58,970| 8,51,942| 8,07,433| 9,06,115| 1,34,234| 2,837    | 71         | 0.56   |
|          | 30    | 14,56,630| 12,32,700| 11,13,220| 12,67,517| 1,74,332| 3,969    | 100        | 0.59   |
|          | 120   | 16,47,590| 12,56,070| 11,46,010| 12,01,040| 77,824    | 3,761    | 95         | 6.5    | 0.81   |

Table 3: Results of TR alpha agonist assays of Metadichol and reference agonist Triiodothyronine.

| Compound | Conc. | Human TRb Antagonist Assays, Host cell line: HEK |
|----------|-------|-----------------------------------------------|
|          |       | Luc1  | Luc2  | Luc3  | AVG  | SD  | Fold-Inhibition | %Inhibition | % CV |
| Water    | 0.20% | 10,00,450| 10,17,700| 10,43,760| 10,20,637| 21,804 | 1 | 0 | 2.1 |
| Metadichol (mg/mL) | 0.0073 | 9,50,092| 9,99,788| 10,31,020| 9,93,633| 40,814 | 1 | 2.6 | 4.1 |
|          | 0.024 | 9,37,815| 10,93,660| 11,11,230| 10,47,568| 95,454 | 0.97 | -2.6 | 9.1 |
|          | 0.081 | 9,08,237| 9,82,324| 10,56,140| 9,82,234| 73,952 | 1 | 3.8 | 7.5 |
|          | 0.27  | 9,37,650| 9,81,457| 10,01,420| 9,73,509| 32,619 | 1 | 4.6 | 3.4 |
|          | 0.9   | 7,85,643| 7,45,300| 7,81,945| 7,70,963| 22,301 | 1.3 | 24 | 2.9 |
|          | 3     | 5,14,370| 4,67,836| 4,85,441| 4,89,216| 23,496 | 2.1 | 52 | 4.8 |
|          | 10    | 1,98,260| 1,89,334| 2,01,774| 1,96,456| 6,413 | 5.2 | 81 | 3.3 |

Table 4: TR alpha antagonist assays of Metadichol and reference agonist Triiodothyronine.

| Compound | Conc. | Human TRb Antagonist Assays, Host cell line: HEK |
|----------|-------|-----------------------------------------------|
|          |       | Luc1  | Luc2  | Luc3  | AVG  | SD  | Fold-Inhibition | %Inhibition | % CV |
| Water    | 0.20% | 8,03,254| 8,07,166| 8,29,297| 8,13,239| 14,044 | 1 | 0 | 1.7 |
| Metadichol (ug/mL) | 0.0073 | 8,71,672| 8,70,226| 9,68,181| 9,03,360| 56,142 | 0.9 | -11 | 6.2 |
|          | 0.024 | 8,61,060| 7,68,219| 8,42,466| 8,23,915| 49,122 | 0.99 | -1.3 | 6 |
|          | 0.081 | 7,74,125| 8,28,213| 8,96,931| 8,33,090| 61,548 | 0.98 | -2.4 | 7.4 |
|          | 0.27  | 7,35,981| 8,31,300| 7,86,600| 7,84,536| 47,556 | 1 | 3.5 | 6.1 |
|          | 0.9   | 6,04,043| 6,26,890| 6,56,405| 6,29,113| 26,252 | 1.3 | 23 | 4.2 |
|          | 3     | 3,53,284| 3,32,801| 4,17,058| 3,67,714| 43,943 | 2.2 | 55 | 12 |
|          | 10    | 89,689 | 1,14,198| 1,08,014| 1,03,966| 12,746 | 7.8 | 87 | 12 |

Table 5: TR beta antagonist assays of Metadichol and reference agonist Triiodothyronine.
Figure 3: Result of TR-alpha assay of standard Triiodothyronine.

Figure 4: Result of TR-alpha agonist assay of Metadichol.

Figure 5: Result of TR-alpha antagonist assay of Metadichol.

Figure 6: Result of TR-beta agonist assay of Metadichol.

Figure 7: Result of TR beta agonist assay of standard Triiodothyronine.

Figure 8: Result of TR-beta antagonist assay of Metadichol.
### Table 6: TR β agonist assays of Metadichol and reference agonist Triiodothyronine.

| Compound | Conc. (ug/mL) | Human TRβ Agonist Assays, Host cell line: HEK |
|----------|--------------|-----------------------------------------------|
| Water    |              | Luc1  Luc2  Luc3  AVG  SD  Fold-Activation  % Activation of Refmax  % CV |
|          |              | 118     132   154   135  18   1            0.012             13 |
| Metadichol | 0.0073       | 124     120   156   133  20   0.99          0.012             15 |
|          | 0.024        | 144     108   142   131  20   0.98          0.012             15 |
|          | 0.081        | 110     134   122   122  12   0.91          0.011             9.8 |
|          | 0.27         | 142     136   120   133  11   0 Compound.99  0.012             8.6 |
|          | 0.9          | 104     120   148   124  22   0.92          0.011             18 |
|          | 3            | 106     80    182   123  53   0.91          0.011             43 |
|          | 10           | 60      68    74    67   7     0.5          0.006             10 |

Reference Agonist: Triiodothyronine (nM)

| Conc. (nM) | Luc1  Luc2  Luc3  AVG  SD  Fold-Activation  % Activation of Refmax  % CV |
|------------|--------|--------|--------|------|----------|----------------------|------|
| 3.3        | 167    172   174  174   2   1.3          0.015             1.1 |
| 9.9        | 2,140  1,942 1,652 1,912 245 14  0.17              13  0.55 |
| 30         | 97,190 95,283 81,040 91,171 8,826 677 8.1  9.7  0.71 |
| 89         | 7,52,651 8,52,420 7,91,517 8,12,196 34,840 6031 7.2  4.3  0.87 |
| 267        | 10,89,770 10,91,930 11,09,530 10,97,077 10,839 8,147 97  1  0.97 |
| 800        | 12,62,650 11,56,450 11,12,770 11,77,357 77,193 6,843 104  6.6  0.8 |
| 2400       | 10,71,220 10,86,870 12,26,570 11,28,220 85,532 8,378 100  7.6  0.77 |

Figure 9: TSH levels (mIU/mL).

Figure 10: Total T3 levels.

Figure 11: Free T4 levels.
Figure 12: Thyroid antibody levels.

Figure 13: Thyroglobulin levels.

|                | TPO (IU/mL) |
|----------------|-------------|
| Baseline       | 20.1        |
| Week 4         | 24          |
| Week 8         | Less than 0.4 |
| Week 12        | Less than 0.4 |
| week 16        | Less than 0.4 |
| week 20        | 20          |
| week 24        | 6           |
| week 32        | less than 0.4 |

Figure 14: TPOab (Thyroid peroxidase antibodies) levels.
Patient No 4: Female-51 (figure 17)

Female 51 diagnosed with hypothyroidism was not on any Thyroid medication. Metadichol® treatment at 5 mg per day. Patient normalized over 20 weeks.

Patient 5: Female-27 (figure 18-19)

Female 27 diagnosed with hypothyroidism. Was not on any thyroid medication. Metadichol treatment @ 5 mg per day improved TSH levels. Patient still on Metadichol. Patient reports improved energy levels (Figures 18 and 19).
Patient No 6: Female-27 (figure 20-21)

Female 27 diagnosed with hyperthyroidism. Her doctor prescribed Carimazol but she opted to use Metadichol. After 4 months all her Thyroid biomarkers returned normal. She stopped Metadichol use at 10 month and a year later at month 22 her biomarkers are still in the normal range.

Discussion

The cases presented all showed improvement in all thyroid biomarkers panel. Metadichol binds to thyroid nuclear receptors both THRA and THRB but as an inverse agonist. There are no known inverse agonists of thyroid nuclear receptors known in literature today. For inverse agonism to exist there must be constitutive activity present i.e. receptors are actively signaling in the absence of an agonist [25]. Related to inverse agonism is what is referred to as protean agonism [26] such ligands display both agonist and inverse agonist properties. Efficacy of a ligand is depending on the level of receptor constitutive activity of the receptor in the assay system used. Therefore, a partial agonist in one system can behave as an antagonist or an inverse agonist in others. For protean agonism to exist there needs to be constitutive receptor activity i.e. inverse agonism that is seen with Metadichol*. The results show that it is effective against both hyperthyroidism and hypothyroidism and normalizing T3, T4 and levels of antibodies. It is likely that Metadichol* is behaving as a protean agonist. Metadichol is also an inverse agonist of other nuclear receptors VDR, AHR (Aryl hydrocarbon receptor) and RORC (RAR related Orphan receptor C) [24,27,28].
With the experimental data of binding to the thyroid receptors, using a web-based program like EviNet [29] to see the network enrichment analysis of the genes of interest. It allows applicability of genes found in global network to connect genes via one various molecular mechanism. Using these just 2 genes THRA and THRB led to over 100 significant pathways and the top 5 are shown in Table 7. From this analysis the enriched Reactome pathways [30] from THRA and THRB are linked to 321 genes in the network. Figure 22 shows the network of genes involved with regulation of thyroid hormone pathway with a FDR (False Discovery Rate) value of 8.6×10^{-292}.

**DIO 1**: It catalyzes the activation, as well as the inactivation of thyroid hormone. Responsible for the deiodination of T4 (3,5,3',5'-tetraiodothyronine) into T3 (3,5,3'-triiodothyronine) and of T3 into T2 (3,3'-diiodothyronine) which is inactive.

**DIO 2**: Catalyzes the conversion T4 to bioactive T3. It has an essential role in providing the brain with appropriate levels of T3 during the critical period of development.

**DIO 3**: Responsible for the deiodination of T4 into RT3 (3,3',5'-triiodothyronine) and of T3 into T2 (3,3'-diiodothyronine). RT3 and T2 are inactive metabolites.

The network clearly shows how genes DIO 1, 2 and 3 play a role in maintaining appropriate levels of T3 and T4 in regulating the thyroid hormone pathway. It is clear that binding to both THRA and THRB is important in enhancing the thyroid pathway. Metadichol’s binding to thyroid nuclear receptors leads to control of TSH and also T3 and T4 levels.

The second pathway that is enriched is transcriptional regulation of white adipocyte differentiation. The adipose tissue is targeted by thyroid hormones as this tissue is where energy stored and acts as a regulator of energy balance, and modulating signals to maintain metabolic control. Thyroid hormones regulate the gene markers of differentiation of adipocytes, lipogenesis, lipolysis, and thermogenesis in the adipose tissue [31].

Research work on use of selective agonist of THRB in lipid related disease though the results have been disappointing [32].

Metadichol also binds to VDR, AHR and ROR gamma as inverse agonists. Given the cross talk that occurs between various nuclear receptors a connected network of nuclear receptors [33] can be generated using a software like Innate DB [34-36] a database of genes, proteins, experimentally-verified interactions and signaling pathways involved in the innate immune response of humans. This is shown in Figure 23. The numbers in the figure are known nuclear receptors. What we see is binding to multiple receptors leads to activating other nuclear receptors in this case 37 out of 48 known in humans. Binding and or activation of these nuclear receptors leads to gene transcription and leads to many pathways and thus many targets. Metadichol has already been shown to impact many diseases through a multitude of pathways [37].

A macro level interaction network of TNF alpha, ICAM1 and the nuclear receptors VDR, THRA, THRB shown in Figure 24. This was generated using pathway commons [38]. VDR controls the expression of THRA that interacts with THRB [39]. A more detailed interaction is shown in Figure 25 generated by using InnateDB software.

| Gene set | Number of links | Enriched pathways | FDR (False Discovery Rate) | Reactome pathway |
|----------|-----------------|-------------------|-----------------------------|-----------------|
| THRA, THRB | 321 | Regulation of Thyroid hormone | 8.60E-292 | R-HSA-350864 |
| THRA, THRB | 321 | Transcriptional regulation of white adipocyte differentiation | 2.20E-112 | R-HSA-381340 |
| THRA, THRB | 321 | Thyroxine biosynthesis | 1.40E-82 | R-HSA-209968 |
| THRA, THRB | 321 | Xenobiotics | 1.60E-61 | R-HSA-211981 |
| THRA, THRB | 321 | CYP2E1-reactions | 3.50E-48 | R-HSA-211999 |

Table 7: Enriched pathways of THRA and THRB generated by EVINET.
Figure 23: Network of Nuclear receptors AHR, VDR, THRA, THRB and RORC.

Figure 24: Interaction network of thyroid related gene cluster.
Figure 25: Interaction Network of TNF, ICAM1, CCL2, VDR, THRA, THRB.

Figure 26: Gene cluster; THRA, THRB, VDR, ICAM1, TNF, CCL2 and the resulting disease network.
Further analysis was carried out using a program like ToppCluster [40] a multiple gene list feature enrichment analyzer for the dissection of biological system. This cluster of six genes (TNF, ICAM1, CCL2, VDR, THRA, THRB) target all the thyroid related diseases as shown in Figure 26. Drugs are more effective if they are modulating multiple genes [41]. All diseases need complex therapeutic approaches. In this respect, as shown above Metadichol ™ hits multiple genes belonging to a network of interacting targets and thus has enhanced and efficacy and an enormous advantage when compared to a single-target drug or a combination of multiple drugs [42,43]. Compared to drugs used in treating diseases Metadichol is a safe and effective nutraceutical to mitigate Thyroid related diseases.

Conclusion
Diseases generally are not the result of a single malfunctioning gene or a single malfunctioning pathway. There are many genes that affect the same disease and need to tackle though multiple pathways. The concept of one gene, one disease and one drug are no longer a viable approach. Thus, there is a need to target nuclear receptors which is the operating system of our body. Binding to vitamin D receptor for example is known to affect over 2900 genes and the same is the case with other nuclear receptors. The reason that we have failed to address many diseases adequately in the past was the lack of information regarding how genes are related to each other. Today a vast amount of information is at our finger tips. The key to every biological problem must finally be a network of interacting targets and thus has enhanced and efficacy. The concept of one gene, one disease and one drug are no longer a viable approach. Thus, there is a need to target nuclear receptors.

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