α-Methylated Analogues of Triiodothyroalkanoic Acids: Synthesis and Biological Activity

N. Zenker,* A. E. Ekpe,† and L. S. Hubbard

Department of Biomedicinal Chemistry, School of Pharmacy, University of Maryland at Baltimore, Baltimore, Maryland 21201. *Received December 14, 1991*

Three novel thyroid hormone analogues: α-methyl-3,5,3′-triiodothyroacetic acid, α-methyl-3,5,3′-triiodothyropropionic acid, and α-methyl-3,5,3′,5′-tetraiodothyropropionic acid were synthesized. The hepatic thyroid receptor affinity of these analogues was compared to that other available thyroid analogues. The ability of these compounds to increase the activity of two hepatic enzymes and to lower blood cholesterol was compared to that of L-triiodothyronine. α-Methyl-3,5,3′-triiodothyroacetic acid was shown to have less nuclear binding affinity, less enzyme inducing ability, but more blood cholesterol lowering ability than triiodothyroacetic acid. α-Methyl-3,5,3′,5′-tetraiodothyropropionic acid showed less nuclear binding affinity and less enzyme-inducing activity than α-methyl-3,5,3′-triiodothyropropionic acid.

Introduction

Thyroid hormone receptor sites with high affinity for L-triiodothyronine (L-T_3) have been identified first in nuclei of pituitary cells[1] and then in nuclei of liver and kidney cells.[2] Since most L-T_3 effects were found to require the binding of L-T_3 to a nuclear receptor, a good correlation between the receptor binding affinity and the thyromimetic activity of thyroid analogues was anticipated and eventually established.[3] Notable exceptions to this correlation were triiodothyroacetic acid (TRIAC) and triiodothyropropionic acid (TRIPROP), two compounds with a nuclear receptor affinity in excess of,[4,5] and a biological activity of less than half of, that of L-T_3.[5,6]

The reason for the disparity between the strong nuclear binding and the relatively weak thyromimetic effects of TRIAC has been extensively investigated by Oppenheimer and co-workers.[4,7] On the basis of the rapid in vivo metabolism of labeled TRIAC, Oppenheimer[4] concluded that the low biological activity of TRIAC was related to its rapid removal from nuclear receptor sites.[7] TRIPROP, which is not considered a metabolite of L-T_3,[8] has not been similarly investigated.

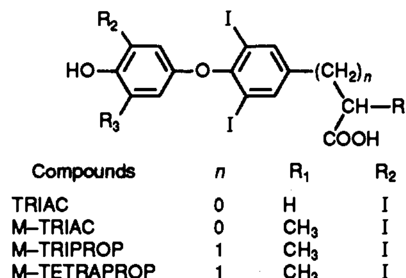
Since the metabolism of the branched chain fatty acids, e.g. those derived from branched-chain amino acids,[9] is more complex and possibly slower than the metabolism of straight-chain acids, we hypothesized that α-methyl-3,5,3′-triiodothryoacetic acid (M-TRIAC) (Chart I), with an α-methylacetic acid side chain, would be metabolized more slowly than the acetic acid side chain bearing TRIAC and would have greater biological activity if the introduction of an α-methyl group would not adversely affect its binding to the receptor.

The first model representing the binding of L-T_3 to a putative receptor[10] emphasized the perpendicularity of the phenyl rings of 3,5-diiodothyronines and postulated that the 3′-substituent in 3′-substituted 3,5-diiodothyronines was oriented in a distal position, i.e., away from the side-chain-bearing ring, but did not describe factors contributing to the binding of the L-T_3 side chain to the receptor. By measuring the receptor affinity of the L-T_3 and L-thyroxine (L-T_4) methyl isoesters α-methyl-3,5,3′-triiodothyropropionic acid (M-TRIPROP) and α-methyl-3,5,3′,5′-tetraiodothyropropionic acid (M-TETRAPROP) (Chart I), respectively, we hoped to contribute to the understanding of the factors involved in the binding of the thyroid hormone side chain to its receptor.

*To whom all inquiries should be addressed.

†Submitted as partial fulfillment for the degree of Doctor of Philosophy, University of Maryland, 1986.

Chart I. Structures of Thyroid Analogues



Compounds	n	R1	R2	R3
TRIAC	0	H	I	H
M-TRIAC	0	CH3	I	H
M-TRIPROP	1	CH3	I	H
M-TETRAPROP	1	CH3	I	I

Table I. Affinity of Test Compounds for Rat Liver Nuclei[a]

test compound	K_i ± SE, nM	rel affinity (% T_3)
L-triiodothyronine	1.56 ± 0.18	100
TRIAC	0.79 ± 0.15	199
M-TRIAC (±) (VII)	9.03 ± 1.2	17
M-TRIPROP (±) (XI)	2.56 ± 0.12	61
M-TETRAPROP (±) (XII)	13.0 ± 1.3	12
α-methyl-T_4	63.3 ± 12.7	2

[a] Rat hepatic nuclear extract, incubated with $[^{125}I]T_3$ (10 nCi) and increasing concentrations of analogue (each incubated in quadruplicate) yielded the IC_{50} (the analogue concentration able to displace 50% of $[^{125}I]T_3$). The K_i of each analogue was obtained from $K_i = IC_{50}/(1 + {*L}/K_d)$,[12] where *L is the concentration of $[^{125}I]T_3$ (0.075 nM) and K_d is the K_i for T_3. The relative nuclear affinity of test compounds, expressed as % T_3 affinity, is the ratio of the K_i of L-T_3 to that of the analogue.

Results and Discussion

The affinity of analogues and reference compounds for the thyroid receptor was estimated in vitro by the com-

(1) Schadlow, A. R.; Surks, M. I.; Schwartz, H. L. *Science* **1972**, *176*, 1252-1254.
(2) Oppenheimer, J. H.; Koerner, D.; Schwartz, H. L. *J. Clin. Endocrinol. Metab.* **1972**, *35*, 330-333.
(3) Koerner, D.; Schwartz, H. L.; Surks, M. I.; Oppenheimer, J. H.; Jorgensen, E. C. *J. Biol. Chem.* **1975**, *250*, 6417-6423.
(4) Oppenheimer, J. H. In *Molecular Basis of Thyroid Hormone Action*; Oppenheimer, J. H., Samuels, H. H., Eds.; Academic Press: New York, 1983; Chapter 1.
(5) DeGroot, L. J., Reed Larsen, P., Refetoff, S.; Stanbury, J. B., Eds. *The Thyroid and Its Diseases*, 5th ed.; Wiley & Sons: New York, 1984; Chapter 2.
(6) Stasilli, N. R.; Kroc, R. L., Meltzer, R. I. *Endocrinology* **1959**, *64*, 62-82.
(7) Goslings, B.; Schwartz, H. L.; Dillmann, W.; Surks, M. I.; Oppenheimer, J. H. *Endocrinology* **1976**, *98*, 666-675.
(8) Cody, V. *Endocr. Res.* **1988**, *14*, 165-176.
(9) Stryer, L. *Biochemistry*, 3rd ed.; W. H. Freeman & Co.: New York, 1988; p 510.
(10) Zenker, N.; Jorgensen, E. C. *J. Am. Chem. Soc.* **1959**, *81*, 4643-4647.

0022-2623/92/1835-0548$03.00/0 © 1992 American Chemical Society

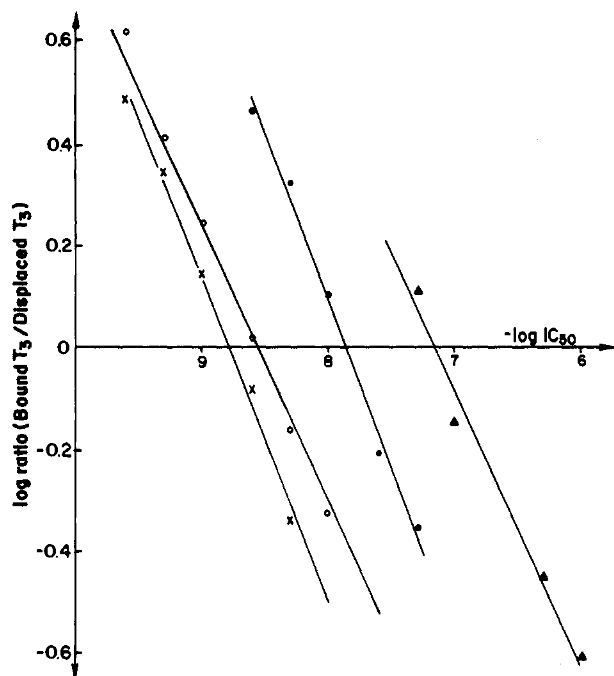


Figure 1. Hill plot of the displacement of [^{125}I]-L-T_3 by increasing doses of thyroid analogues. [^{125}I]-L-T_3 (10 nCi, 0.75 pmol, in 0.1 mL) was incubated with a hepatic nuclear fraction from thyroidectomized animals (in 0.8 mL) and analogue in given concentrations (in 0.1 mL). Total binding was determined in the absence of unlabeled T_3 and nonspecific binding by incubation in the presence of 1 μM unlabeled T_3. Each point was assayed in quadruplicate. The data are plotted as the log of the ratio of [^{125}I]-L-T_3 bound to [^{125}I]-L-T_3 displaced vs the negative logarithm of the dose of analogue administered. The analogues represented are L-T_3 (×), M-TRIPROP (○), M-TETRAPROP (●), and M-T_4 (▲). The x intercept (log ratio = 0) yield the negative logarithm of the IC_{50}.

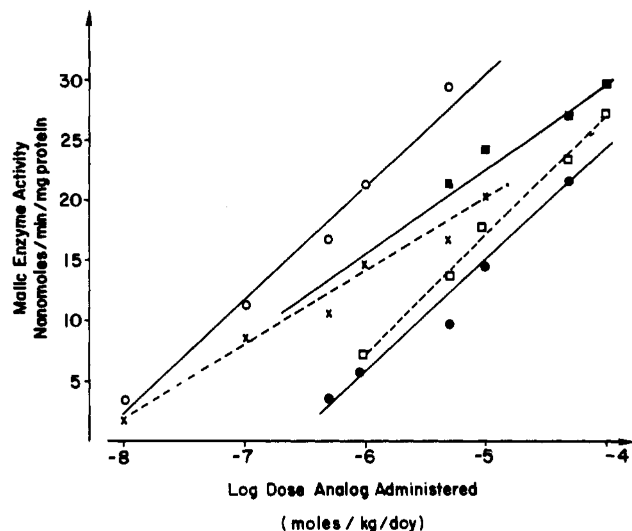


Figure 2. Effect of analogue administration on hepatic malic enzyme activity. Malic enzyme activity of the hepatic supernatant of thyroidectomized animals administered L-T_3 (○), M-TRIPROP (×), M-TETRAPROP (●), M-TRIAC (□), or TRIAC (■) analogue for 3 days. Enzyme activity is expressed in nmol NADPH reduced/min per mg protein vs the dose of analogue administered. The activity of controls was 1.68 ± 0.07 (σ). There were two animals at each dose point and each assay was performed in duplicate.

petitive displacement of radiolabeled (^{125}I) L-T_3 from rat hepatic nuclei by the analogue to be tested.[3] The concentration of a given analogue able to displace 50% of the radiolabel from nuclear receptors, referred to as IC_{50}, was obtained from a Hill plot[11] of the displacement curve (Figure 1). From the IC_{50} value the equilibrium dissociation constant K_i of the analogue tested was then calculated;[12] relative affinity was expressed as the ratio of the K_i value of L-T_3 to that of the analogue.

The K_i values obtained in this study are presented in Table I. The K_i value of 1.56 nM (obtained from an IC_{50} value of 1.66 nM) for L-T_3 is well in the range of the IC_{50} of 2.8 nM that Koerner et al.[3] obtained in vitro with hepatic cell nuclei and the K_i of 0.84 nM calculated from data obtained with a solubilized receptor system.[13] The relative affinity of TRIAC is, at 199% L-T_3, well within the range of 160–400% L-T_3 published elsewhere.[4,5]

The data of Table I indicate that the introduction of an α-methyl group in TRIAC reduced its relative receptor affinity from 199 to 17% L-T_3. The data of Table I indicate also that the relative affinity of racemic M-TETRAPROP (12% that of L-T_3) is much smaller than that of racemic M-TRIPROP (61% that of L-T_3), an observation consistent with the lower affinity of TETRAC vs TRIAC[4] and of L-T_4 vs L-T_3.[4,13]

Since TRIPROP was found to have more than twice the affinity of L-T_3[13] and since our results indicate that racemic M-TRIPROP has 61% the affinity of L-T_3, it may be suggested that, with reference to binding at the hepatic nuclear receptor site, α-methyl and α-amino groups both lower the binding affinity of TRIPROP. The implications of this finding are, first, that the positive charge of the amino group of the iodothyronines does not play a role in binding to the nuclear receptor. Further, that the decreased affinity seen when comparing L-T_3 to TRIPROP does not relate to the acid strength of the carboxyl group insofar as the dissociation constants of the TRIPROP and M-TRIPROP carboxyl groups are similar but significantly smaller than that of L-T_3 (the pK of phenylpropionic acid is 4.7, that of diiodotyrosine, 2.1). Finally, this finding provides an example of the validity of isosteric considerations in receptor ligand interactions.

A comparison of the relative affinity of α-methylthyroxine (2% L-T_3), an analogue which has both an α-methyl and an α-amino substituent, with that of M-TETRAPROP (12% L-T_3) indicates that both the α-amino and the α-methyl group sterically restrict the binding of the analogue to the hepatic nuclear receptor. The receptor affinities of M-TRIAC, of M-TRIPROP, and of α-methylthyroxine all indicate that steric constraints play an important role in receptor binding.

Increases in the activity of α-glycerophosphate dehydrogenase (GPD) and of malic enzyme (ME), which have been recognized as thyromimetic effects for some time,[14,15] have more recently been proven to be induced by the interaction of thyroid hormone with its nuclear receptor.[16] Guernsey and Edelman[17] have pointed to the excellent

(11) Hill, A. V. *Biochem. J.* **1913**, *7*, 471–480.
(12) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(13) Bolger, M. B., Jorgensen, E. C. *J. Biol. Chem.* **1980**, *255*, 10271–10278.
(14) Ruegamer, W. R., Westerfeld, W. W.; Richert, D. A. *Endocrinology* **1964**, *75*, 908–916.
(15) Wise, E. M.; Ball, E. G. *J. Biol. Chem.* **1964**, *175*, 385–390.
(16) Dillmann, W. H.; Schwartz, H. L.; Silva, E.; Surks, M. I.; Oppenheimer, J. H. *Endocrinology* **1977**, *100*, 1621–1627.
(17) Guernsey, D. L.; Edelman, I. S. In *Molecular Basis of Thyroid Hormone Action*; Oppenheimer, J. H., Samuels, H. H., Eds.; Academic Press: New York, 1983; Chapter 10.

Table II. Enzyme Induction by Thyroid Analogues[a]

	GPD activity			ME increase		
	nmol/min per mg		as % L-T_3	nmol/min per mg		as % L-T_3
	10^{-6}	10^{-5}		10^{-6}	10^{-5}	
L-T^3	7.5	18.3	100	19.5	31.8	100
TRIAC	$4.8^{c,d}$	$12.9^{c,d}$	69	13.0	$22.5^{c,d}$	69
M-TRIAC (VII)	1.5	6.6	31	5.6	15.5	41
TRIPROP	2.4	8.6	43	5.5	12.9	36
M-TRIPROP (XI)	$7.8^{e,f}$	12.1^{e}	77	$13.1^{e,f}$	$18.6^{e,f}$	62
TETRAPROP (XII)	1.8	7.4	36	6.8	14.7	42

[a] Hepatic GPD and ME activity increases (above controls) of thyroidectomized animals injected for 3 days with 10^{-6} and 10^{-5} mol/kg doses of T_3 or thyroid analogue. There were two animals for each test point and each analysis was performed in duplicate. The results are expressed in nmol/min per mg of protein. For GPD the controls were 6.18 ± 0.25 and the average precision of duplicates was 2.4%. For ME the controls were 1.68 ± 0.07 and the average precision was 1.2%. Values significantly greater at $P < 0.05$. [b] T_3 vs TRIPROP. [c] TRIAC vs M-TRIAC. [d] TRIAC vs M-TRIPROP. [e] M-TRIPROP vs M-TETRAPROP. [f] M-TRIPROP vs M-TRIPROP.

correlation of GPD activity and the calorigenic effect; hepatic ME has been used as a marker of thyroidal status in the rat,[18] and both GPD and ME activity measurements have been used as indices of biological activity in thyroid analogues.[19-22]

The hepatic GPD and ME activity of rats administered 10^{-4}–10^{-8} M daily doses of analogue for 3 days increased total GPD and ME activity roughly in proportion to the logarithm of the dose of analogue tested (Figure 2). The inducing effect of 10^{-5} and 10^{-6} M doses of M-TRIAC, M-TRIPROP, and M-TETRAPROP is compared in Table II with those of L-T_3, TRIAC, and TRIPROP. Statistical consideration of the data (Table II) suggests that the order of inducing effectiveness of the compounds tested is L-T_3 > TRIAC and M-TRIPROP > TRIPROP > M-TRIAC and M-TETRAPROP.

The relative order of L-T_3 > TRIAC > TRIPROP is in agreement with other data obtained for these compounds.[6,23] The relative order of M-TRIPROP > M-TETRAPROP is consistent with the relative biological activities of L-T_3 and L-T_4 and of TRIPROP and TETRAPROP.[6] It was of interest that α-methyl substitution lowered the inducing effectiveness of TRIAC but increased that of TRIPROP. The search for hypocholesteremic agents has long been a key incentive for the synthesis of thyroid hormone analogues. Early efforts focused on D-triiodothryonine,[24] on newly synthesized 3,5-diiodotyrosine ethers,[25] and on a number of thyroid hormone analogues with modified side chains.[26] More recently investigated hypocholesteremic thyroid analogues include the presumably less cardiotoxic 3'-isopropyl-3,5-diiodothyroacetic acid,[27,28] which had been previously synthesized,[29] and a large series of mainly 3'-(methylaryl)-3,5-dihalothyronines,[20,21,30,31] which were tested for hepatic and cardiac effects in an effort to develop hypocholesteremic agents with minimal cardiac side effects.

Table III. Hypocholesteremic Effects of Some α-Methylated Analogues

dose, nmol/kg	L-T_3	M-TRIAC (+)	M-TRIAC (±)	TRIPROP
4	251 ± 69	262 ± 53	259 ± 58	nd
8	138 ± 71^{b}	147 ± 61^{b}	216 ± 37^{b}	285 ± 59
16	101 ± 34^{c}	119 ± 34^{c}	nd	202 ± 58^{b}

[a] Normal Sprague–Dawley rats weighing 265 ± 10 g were placed on a pellet diet containing 2% cholesterol and 1% cholic acid for 7 days while receiving daily subcutaneous injections of analogue in doses expressed in nmol/kg. Twenty-four hours after the last injection, the animals were sacrificed and their trunk blood analyzed. There were five animals in each group, the results are expressed in mg/dL ± SD. The cholesterol level of animals receiving vehicle only was 285 ± 63 mg/dL. [b] $P < 0.01$. [c] $P < 0.001$ vs cholesterol diet control.

The effect of the administration of nanomole/kilogram doses of L-T_3, M-TRIAC, and M-TRIPROP on the plasma cholesterol levels of animals on a cholesterol–cholic acid diet is presented in Table III. The dose of analogue needed to lower plasma cholesterol to half the control level was obtained from a milligram/deciliter cholesterol vs L-T_3 log dose regression line[24] and was then converted to relative effectiveness (% L-T_3): for L-T_3 that dose was 10.4 nM; for (+) M-TRIAC, 11.6 nM (90% L-T_3); for racemic M-TRIAC, 18.6 nM (56% L-T_3); and for racemic M-TRIPROP, 32.3 nM (32% L-T_3). These findings suggest that M-TRIAC and possibly also M-TRIPROP are more effective plasma cholesterol lowering agents than TRIAC (30% L-T_3)[26] and TRIPROP,[32] respectively. The cardiac effect of these compounds was not investigated.

Some of the results obtained in this study conform with previous statements on the correlation between the receptor affinity and the biological activity of thyroid hormone analogues.[3] Thus the comparison of the enzyme-inducing activity of TRIAC with that of M-TRIAC suggests that the decreased inducing ability of the latter may be linked to its 10-fold decrease in receptor affinity. Further, M-TRIPROP has more affinity and more activity than M-TETRAPROP, and the marginal hepatic GPD induction[19] caused by α-methylthyroxine could have been anticipated from its low affinity. However, other observations indicate that factors other than receptor affinity affect biological activity. Thus the introduction of an α-methyl group in TRIPROP led to a decline in receptor affinity but to an increase in both its inducing effectiveness

(18) Towle, H. C. In *Molecular Basis of Thyroid Hormone Action*; Oppenheimer, J. H., Samuels, H. H., Eds.; Academic Press: New York, 1983; Chapter 6.
(19) Zenker, N.; Truchot, R.; Goudonnet, H.; Chaillot, B.; Michel, R. *Biochem. Pharmacol.* **1976**, *25*, 1757–1762.
(20) Benson, M. G.; Ellis, D.; Emmett, J. C.; Leeson, P. D.; Pearce, N. J.; Shah, V. P.; Underwood, A. H. *Biochem. Pharmacol.* **1984**, *33*, 3143–3149.
(21) Leeson, P. D.; Ellis, D.; Emmett, J. C.; Shah, V. P.; Showell, G. A.; Underwood, A. H. *J. Med. Chem.* **1988**, *31*, 37–54.
(22) Goodridge, A. G.; Garay, A.; Silpananta, P. *J. Biol. Chem.* **1974**, *249*, 1469–1475.
(23) Pittman, C. S.; Barker, S. B. *Am. J. Physiol.* **1959**, *197*, 1271.
(24) Greenberg, C. M.; Bocher, C. A.; Kerwin, J. F.; Greenberg, S. M.; Lin, T. H. *Am. J. Physiol.* **1961**, *201*, 732–736.
(25) Jorgensen, E. C.; Lehman, P. A.; Greenberg, C.; Zenker, N. *J. Biol. Chem.* **1962**, *237*, 3832–3838.
(26) Greenberg, C. M.; Blank, B.; Pfeiffer, F. R.; Pauls, J. F. *Am. J. Physiol.* **1963**, *205*, 821–826.
(27) Loireau, A.; Autissier, N.; Dumas, P.; Michel, O.; Jorgensen, E. C. *Biochem. Pharmacol.* **1986**, *35*, 1691–1696.
(28) Michel, R.; Vendel, J. R.; Bourquin, C. *Chem. Abstr.* **1982**, *97*, 222988v. Michel, R., Eur. Pat. Appl. EP (Cl. C07C59/68), 1982.
(29) Blank, B.; Pfeiffer, F. R.; Greenberg, C. M.; Kerwin, J. F. *J. Med. Chem.* **1963**, *6*, 560–563.
(30) Underwood, A. H.; Emmett, J. C.; Ellis, D.; Flynn, S. B.; Leeson, P. D.; Benson, G. M.; Novelli, R.; Pearce, N. J.; Shah, V. *Nature* **1986**, *324*, 425–429.
(31) Leeson, P. D.; Emmett, J. C.; Shah, V. P.; Showell, G. A.; Novelli, R.; Prain, H. D.; Benson, M. G.; Ellis, D.; Pearce, N. J.; Underwood, A. H. *J. Med. Chem.* **1989**, *32*, 320–336.
(32) Greenberg, C. M.; Mansor, L. F.; Bocher, C. A.; Saunders, H. L.; Kerwin, J. F. *Endocrinology* **1962**, *70*, 365–374.

and hypocholesteremic effect, suggesting that the metabolism of M-TRIPROP might possibly be slower than that of TRIPROP. Other observations suggest that the complex factors regulating hypocholesteremic effects[30] may not be as closely related to receptor affinities as are the inducing effects. Thus the hypocholesteremic effect of racemic M-TRIPROP (32% L-T_3 or 43% DL-T_3[24]) is less than could be anticipated from its receptor affinity (61%). Further, no explanation can be offered for the hypocholesteremic effectiveness of M-TRIAC (Table III), or of its more potent (+)-enantiomer (Table III) which did not differ from the M-TRIAC racemate in affinity or enzyme induction (data not presented).

Experimental Section

Chemistry. All compounds, made by well-described procedures, were characterized by melting point (uncorrected; Thomas Hoover apparatus), nuclear magnetic resonance spectroscopy (JOELCO-C-60 HL), and electron impact mass spectroscopy (Extrel EL-1000). Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN).

In all cases, an ethyl (4-hydroxy-3,5-diiodophenyl)alkanoate was condensed with bis(4-methoxyphenyl)iodonium iodide (dianisyliodonium iodide)[33] to yield an ethyl [4-(4′-methoxyphenoxy)-3,5-diiodophenyl]-α-methylalkanoate. The protecting groups of the latter intermediate were then hydrolyzed and the resulting diiodothyroalkanoic acid was then iodinated.[34]

I. α-Methylthyroacetic Acid. α-Methyl-4-nitrophenylacetic acid [2-(4-nitrophenyl)propionic acid (Aldrich Chemical Co.)] was reduced in the presence of 10% Pd/C to 4-amino-α-methylphenylacetic acid (I), mp 131–132 °C (EtOH) (lit.[35] mp 128 °C). I was then converted to 4-hydroxy-α-methylphenylacetic acid (II) using the conditions described by Wilkinson,[36] mp 129 °C (C_6H_6) (lit.[37] mp 130 °C).

4-Hydroxy-3,5-diiodo-α-methylphenylacetic Acid (III). Compound II (1.66 g, 0.01 mol), iodinated with I_2 and KI,[34] yielded 3.7 g (88%) of III, mp 147–148 °C (H_2O, MeOH). Anal. ($C_9H_8I_2O_3$) C, H, I.

Ethyl 4-Hydroxy-3,5-diiodo-α-methylphenylacetate (IV). Compound III (2.6 g, 6.2 mmol) refluxed overnight with EtOH (100 mL) and p-toluenesulfonic acid (0.5 g) yielded 2.7 g (93%) of IV, mp 89–90 °C (95% EtOH). Anal. ($C_{12}H_{14}I_2O_3$) C, H, I.

Ethyl 4-(4′-Methoxyphenoxy)-3,5-diiodo-α-methylphenylacetate (V). Dianisyliodonium iodide (D) was prepared in 33% yield, mp 179–182 °C (lit.[38] mp 184 °C). A mixture of IV (0.011 mol) and D (0.028 mol) was stirred for 24 h with MeOH (120 mL), copper powder (1.6 g), and triethylamine (1.6 g)[38] and then filtered, evaporated under reduced pressure, and taken up in C_6H_6. The C_6H_6 was washed (1 N HCl, 1 N NaOH, H_2O, and 5% HOAc), dried (Na_2SO_4), and concentrated. The concentrate, applied to an Al_2O_3 column and eluted with 300 mL of C_6H_6, gave 1.74 g (30%) of V, mp 69–71 °C. Anal. ($C_{18}H_{18}I_2O_4$) C, H, I.

4-(4′-Hydroxyphenoxy)-3,5-diiodo-α-methylphenylacetic Acid (VI). A solution of V (3 mmol) in 100 mL of 1:1 HOAc and HI was refluxed for 4 h and poured into ice–water. The precipitate was filtered, yielding 1.3 g (85%) of VI: mp 182–184 °C; ^{1}H NMR ($CDCl_3$) δ 1.4 (d, 3, J = 8 Hz), 3.8 (q, 1, J = 8 Hz), 6.7 (d of d, 4, J = 8 Hz), 7.8 (s, 2). Anal. ($C_{15}H_{12}I_2O_4$) C, H, I.

4-(3′-Iodo-4′-hydroxyphenoxy)-3,5-diiodo-α-methylphenylacetic Acid (α-Methyl-3,5,3′-triiodothyroacetic Acid, M-TRIAC, VII). A solution of VI (2 mmol) in MeOH (40 mL) and NH_4OH (40 mL) was iodinated with 3.54 mL of 1 N iodine solution at room temperature. After removing the volatile components, the solution was warmed to 60 °C and brought to pH 4.5 with HOAc. The oil which separated was solubilized (10% NaOH) and reprecipitated (10% HCl) to yield 1.05 g (81.7%) of VII: mp 98–102 °C (MeOH–H_2O); ^{1}H NMR [$(CD_3)_2SO$] δ 1.3 (d, 3, J = 8 Hz), 3.05 (s, 1), 3.6 (q, 1, J = 6 Hz), 6.6 (d, 1, J = 3 Hz), 6.8 (s, 1), 7.0 (t, 1, J = 4 Hz), 7.8 (s, 2); MS (70 ev) m/e (rel intensity) 636 (40), 510 (20), 337 (25), 254 (12), 210 (20), 127 (100), 105 (18), 90 (15), 75 (12), 63 (25). Anal. ($C_{15}H_{11}I_3O_4$) C, H, I.

Quinine base (2 mmol) was added to VII (2 mmol) in hot acetone (50 mL)[39,40] and left standing for 12 h. The separated lower oil layer and the supernatant were each triturated (10 mL, 1 N NaOH) and acidified (10% HCl). The supernatant yielded VII (+), $[\alpha]_D$ = +55.5° (c = 0.09, MeOH), mp 101–103 °C, with the same ^{1}H NMR peaks as the unresolved acid; the $[\alpha]_D$ of the oil layer was −55.5°.

II. α-Methylthyropropionic Acids. p-Hydroxy-α-methylcinnamic acid[34] was catalytically reduced to β-(p-hydroxyphenyl)-α-methylpropionic acid and iodinated to β-(3,5-diiodo-4-hydroxyphenyl)-α-methylpropionic acid (VIII), mp 100–102 °C (lit.[34] mp 102 °C). Esterification of VIII (46 mmol) yielded 19.1 g (91%) of ethyl β-(3,5-diiodo-4-hydroxyphenyl)-α-methylpropionate (IX), mp 53–55 °C (EtOH, H_2O). Anal. ($C_{12}H_{14}O_3I_2$) C, H, I.

β-[4-(4′-Hydroxyphenoxy)-3,5-diiodophenyl]-α-methylpropionic Acid (X). Coupling of IX (13 mmol) with D (36 mmol) proceeded as described for V. The residue of the C_6H_6 extract was triturated with petroleum ether and filtered, and the residue refluxed for 4 h in 60 mL of 1:1 HOAc–HI and was poured into 400 mL ice–water containing 1 g $NaHSO_3$, yielding 2 g (28%) of X, mp 173–176 °C (H_2O, AcOH). Anal. ($C_{16}H_{14}I_2O_4$·0.5HOAc) C, H, I.

β-[4-(3′-Iodo-4′-hydroxyphenoxy)-3,5-diiodophenyl]-α-methylpropionic Acid (α-Methyl-3,5,3′-triiodothyropropionic Acid, M-TRIPROP, XI). Compound X (1.7 mmol), iodinated (3.2 mL of 1 N I_2) as described for VII, yielded 92% of XI: mp 135–137 °C (50% MeOH); ^{1}H NMR [$(CD_3)_2SO$] δ 0.9 (d, 3, J = 4 Hz), 2.5 (m, 3), 6.8 (d, 1, J = 3 Hz), 6.9 (s, 1), 7.0 (t, 1, J = 4 Hz), 7.8 (s, 2); MS (70 eV) m/e (rel intensity) 650 (20), 606 (22), 578 (30), 524 (23), 480 (20), 451 (20), 323 (40), 254 (20), 197 (60), 127 (100), 88 (20), 63 (30). Anal. ($C_{16}H_{13}I_3O_4$) C, H, I.

β-[4-(3′,5′-Diiodo-4′-hydroxyphenoxy)-3,5-diiodophenyl]-α-methylpropionic Acid (α-Methyl-3,5,3′,5′-tetraiodothyropropionic Acid, M-TETRAPROP, XII). Compound X (0.95 mmol), iodinated (3.9 mL, 1 N I_2) and processed as described previously, yielded a quantitative amount of XII: mp 168–170 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 1.0 (d, 3, J = 4 Hz), 2.6 (m, 3), 3.3 (s, 1), 7.2 (s, 2), 7.8 (s, 2); MS (70 eV) m/e (rel intensity) 776 (1), 472 (10), 254 (30), 127 (100), 90 (15), 63 (28). Anal. ($C_{16}H_{12}I_4O_4$) C, H, I.

Biological Methods. Sprague–Dawley male rats were used in all experiments: thyroidectomized rats obtained from Zivic-Miller (Pittsburgh, PA) were used in the receptor-displacement study and in the enzyme-induction experiment; normal rats obtained from Dominion Labs (Dublin, VA) were used in assessing hypocholesteremic effects.

Purchased were the following: from Crescent Chemical Co., the compound Hoechst 33258 (a fluorescent agent for DNA determinations); from Bio-Rad Laboratories, a Bio-Rad Protein Assay kit; from Nutritional Biochemicals, Inc., a cholesterol–cholic acid diet; from Electro Nucleonics, Inc., a Gemeni assay kit for cholesterol analyses; from Calbiochem, dithiothreitol (DTT); and from the Sigma Chemical Co., L-triiodothyronine, triiodothyroacetic acid, triiodothyropropionic acid, and all other biochemical reagents. [^{125}I]-L-Triiodothyronine (1200 μCi/μg) was obtained from the New England Nuclear Corporation and α-methylthyroxine [3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-2-methyl-alanine] was a gift from Chemie Gruenenthal, Gmbh.

The ability of analogues to displace [^{125}I]-L-T_3 from hepatic nuclear receptors was taken as index of receptor affinity. The hepatic nuclear fraction, prepared according to the procedure of Buergi and Abbueh,[41] was suspended in a medium of 0.25 M sucrose containing 1.1 mM $MgCl_2$, 2 mM EDTA, and 5 mM DTT

(33) Ziegler, H.; Marr, C. J. Org. Chem. 1962, 27, 3335–3336.
(34) Papa, D.; Schwenk, E.; Breiger, H.; Peterson, V. J. Am. Chem. Soc. 1950, 72, 2619–2623.
(35) Trinius, B. J. Justus Liebigs Ann. Chem. 1885, 227, 267.
(36) Wilkinson, J. H. Biochem. J. 1956, 63, 601–605.
(37) Bougault, M. Ann. Chim. 1902, 25, 519, 530.
(38) Beringer, M. F.; Drexler, M.; Grindler, E. M.; Lumpkin, C. C. J. Am. Chem. Soc. 1953, 75, 312.
(39) Raper, H. S. J. Chem. Soc. 1923, 123, 2558–2559.
(40) Levene, P. A.; Mikesha, L. A.; Passath, K. J. Biol. Chem. 1930, 88, 27–59.
(41) Buergi, U.; Abbuehl, U. Metabolism 1984, 33, 326–328.

and buffered with 20 mM Tris to pH 7.85. The suspension was shown by fluorometric assay[42] to contain 83 μg of DNA/mL. In the nuclear receptor displacement assay, carried out according to the procedure of Samuels and Tsai,[43] the assay tubes contained 0.8 mL of hepatic nuclear fraction, 0.1 mL of [^{125}I]-L-T_3 (10 nCi diluted with unlabeled T_3 to a final concentration of 0.075 nM), and 0.1 mL of test compounds (dissolved in a minimum volume of 0.25 N NaOH and diluted to volume with saline) in concentrations varying from 0.025 to 100 nM. Total binding to the receptor was determined by incubation in the absence of unlabeled T_3 and nonspecific binding by incubation in the presence of 1 μM unlabeled T_3. Each compound was tested at five to eight different concentrations and each test point was assayed in quadruplicate.

For the enzyme-induction assays thyroidectomized male rats (180-200 g) were injected intramuscularly for 3 consecutive days with vehicle or with solutions of compounds at doses varying from 10^{-4} to 10^{-8} mol/kg. Each compound was injected in six test doses and two animals were used at each dose. Twenty-four hours after the last injection the animals were sacrificed and their livers excised and homogenized in 9 volumes of 0.25 M sucrose containing 1 mM EDTA and buffered with 100 mM Tris to pH 7.5. The cytoplasmic fraction of each homogenate was obtained by centrifuging the homogenate (2000g for 10 min and 40000g for 15 min). Total GPD activity was assayed by following the reduction of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium in the whole homogenate.[20,44] ME activity was assayed in the cytoplasmic fraction by following the reduction of NADP+ at 340 nm. The protein content of homogenates and cytoplasmic fractions was assayed by the method of Bradford.[45]

To test for hypocholesteremic effect, rats (in groups of five) were injected daily for 7 days with doses of test compounds varying from 4 to 16 nmol/kg per day while receiving a diet containing 2% cholesterol and 1% cholic acid.[23] At the end of that period the animals were fasted for 18 h and their trunk blood collected in heparinized tubes. Total plasma cholesterol was assayed by a cholesterol-esterase cholesterol-oxidase peroxidase method.

(42) Labarca, C.; Paigen, K. *Anal. Biochem.* **1980**, *102*, 344-352.
(43) Samuels, H. H.; Tsai, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3488-3492.
(44) Fried, G. H.; Greenberg, N.; Antopol, W. *Proc. Soc. Exp. Biol. Med.* **1961**, *107*, 523-525.
(45) Bradford, M. *Anal. Biochem.* **1976**, *72*, 248-254.
(46) Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; Pu, P. C. *Clin. Chem.* **1974**, *20*, 470-475.

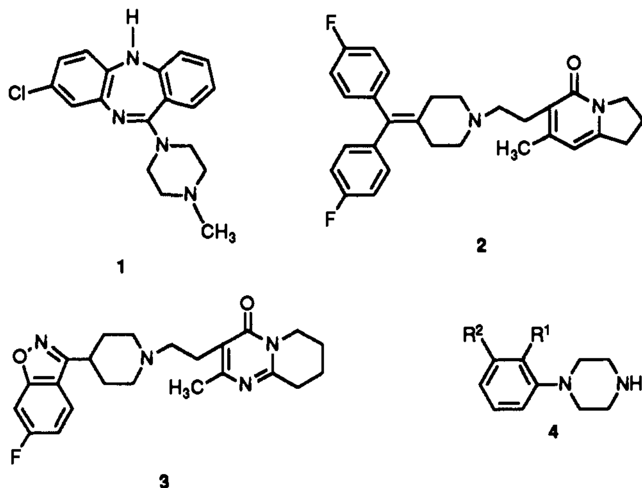
Pyrrole Mannich Bases as Potential Antipsychotic Agents

Malcolm K. Scott,*,† Gregory E. Martin,‡,§ Deena L. DiStefano,† Cynthia L. Fedde,† Michael J. Kukla,† Donna L. Barrett,† William J. Baldy,‡ Robert J. Elgin, Jr.,‡ James M. Kesslick,‡ Joanne R. Mathiasen,‡ Richard P. Shank,‡ and Jeffry L. Vaught‡

Departments of Biological Research and Medicinal Chemistry, McNeil Pharmaceutical, The R. W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477. *Received June 14, 1991*

Recently, we reported on a series of arylpiperazines 4 which exhibit high affinity for the serotonin 5-HT-1A and 5-HT-1B binding sites. Although these compounds interact weakly with dopamine D-1 and D-2 receptors, they are reasonably potent in inhibiting conditioned avoidance responding (CAR) in the rat, an indication of potential antipsychotic activity. Conversion of these arylpiperazines to pyrrole Mannich bases has provided a series of compounds (10-44) which exhibit potent inhibition of CAR when given po and have strong affinity for both the D-2 and 5-HT-1A binding sites. Some of these agents also fail to produce catalepsy. The D-2 binding data and the block of CAR suggest that they are potential antipsychotic agents and the lack of cataleptogenic potential suggests some might possess less liability for producing extrapyramidal side effects and tardive dyskinesias in man.

In the quest for an antipsychotic agent superior to clozapine (1) which does not cause extrapyramidal side effects



(EPS), recent efforts have focused on compounds which interact with the serotonin receptor. Examples of such agents are ritanserin (**2**)[1] and risperidone (**3**),[2] which are potent serotonin-2 receptor (5-HT-2) blockers. Although the blockade of 5-HT-2 receptors has been inferred as a mechanism for reducing EPS,[2] it is not known whether this action contributes to antipsychotic efficacy. Previously, we reported on a series of aryl substituted phenylpiperazines (**4**) with an in vivo profile of activity predictive of antipsychotic activity in man.[3] These compounds are unique, however, insofar as they block conditioned avoidance responding (CAR) in the rat, but do not demonstrate high affinity for the dopamine D-2 binding site in ligand

* Author to whom correspondence should be addressed.
† Department of Medicinal Chemistry.
‡ Department of Biological Research.
§ Present address: Rhône-Poulenc Rorer Central Research, 680 Allendale Rd, King of Prussia, PA 19406.

(1) Reyntjens, A.; Gelders, Y. G.; Hoppenbrouwers, M. L. J. A.; Van Den Bussche, G. Thymosthenic effects of ritanserin (R 55667), a centrally acting serotonin-S_2 receptor blocker. *Drug Dev. Res.* **1986**, *8*, 205-211.
(2) Janssen, P. A. J.; Niemegeers, C. J. E.; Awouters, F.; Schellekens, K. H. L.; Megens, A. A. H. P.; Meert, T. F. Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S_2 and dopamine-D_2 antagonistic properties. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 685-693.
(3) Martin, G. E.; Elgin, R. J., Jr.; Mathiasen, J. R.; Davis, C. B.; Kesslick, J. M.; Baldy, W. J.; Shank, R. P.; DiStefano, D. L.; Fedde, C. L.; Scott, M. K. Activity of aromatic substituted phenylpiperazines lacking affinity for dopamine binding sites in a preclinical test of antipsychotic efficacy. *J. Med. Chem.* **1989**, *32*, 1052-1056.

0022-2623/92/1835-0552$03.00/0 © 1992 American Chemical Society