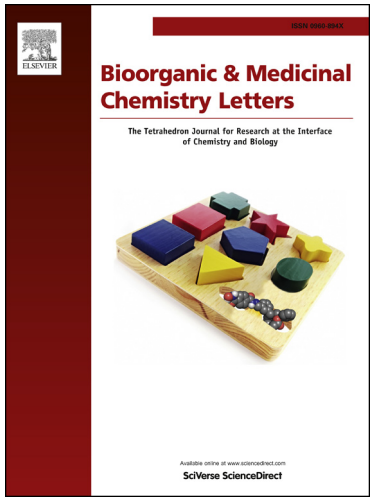

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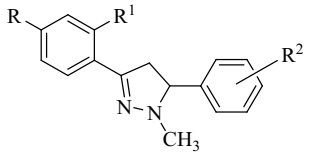

R = H; OH;
R^1 = H; OH;
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Design, Synthesis, and in vitro hMAO-B Inhibitory Evaluation of Some 1-Methyl-3,5-diphenyl-4,5-dihydro-1*H*-pyrazoles

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ABSTRACT

A series of 1-methyl-3,5-diphenyl-4,5-dihydro-1*H*-pyrazoles (**3a-k** and **4a-u**) were designed, synthesized, and evaluated for their inhibitory efficacy towards the two hMAO isoforms. Most of the derivatives were found to be potent and selective hMAO-B inhibitors. In particular, derivative **3g** showed greater hMAO-B affinity than selective inhibitor selegiline coupled with high selectivity index (SI = 145). The most selective hMAO-B inhibitor was the 3-methyl analogue **3f** with an SI higher than 909.

The monoamine oxidases (MAOs) are flavoenzymes located at the outer membrane of mitochondria in the human brain and peripheral tissues. MAOs appear as two isozymes, MAO-A and B, distinguished by substrate and inhibitor selectivities. MAOs catalyze the oxidative deamination of xenobiotic amines and neurotransmitters and play an important role in the central nervous system and peripheral organs. MAO-A metabolizes serotonin, epinephrine and norepinephrine and inhibitors of this isoform are used in the treatment of anxiety disorders and depressive illness.[1] MAO-B is considered to be a major dopamine metabolizing enzyme and its inhibitors reduce the catabolism of dopamine in the basal ganglia and are employed in the symptomatic therapy of Parkinson's disease.[2,3] In Parkinson's disease, MAO-B inhibitors not only prolong the action of dopamine in the brain, but also enhance dopamine levels after treatment with levodopa, the metabolic precursor of dopamine.[4,5] Furthermore, MAO-B inhibitors protect against the neurodegenerative processes associated with Parkinson's disease,[6] decreasing the formation of dopanal and H_2O_2 that arise from the MAO-B catalyzed oxidation of dopamine.[7–11]

Based on the therapeutic implications of MAO inhibitors, the search for novel and selective molecules continues to be an active area of research in medicinal chemistry. Several nitrogen containing heterocycles have been employed in the design of MAO inhibitors. In particular, pyrazole derivatives not only possess biological activities such as antimicrobial,[12] analgesic,[13] antiinflammatory[14] and anticancer[15] but also potent anti-MAO efficacy.[16]

In the context of our search aimed at identifying potent MAO inhibitors,[17-19] we hereby report the synthesis and biological evaluation of some 1-methyl-3,5-diphenyl-4,5-dihydro-1*H*-pyrazoles (Figure 1).

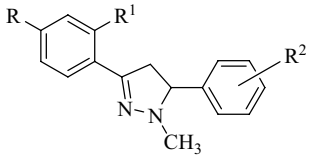

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Figure 1. 1-Methyl-3,5-diphenyl-4,5-dihydro-1*H*-pyrazoles

Derivatives **3a-k** and **4a-u** were synthesized as reported in Scheme 1. The intermediate chalcones **1a-k** and **2a-u** were prepared by base-catalyzed condensation of substituted benzaldehydes with the appropriate acetophenone, eventually followed by acid deprotection of the *p*-hydroxyl group (**2a-u**). Further reaction of the chalcones **1a-k** and **2a-u** with methylhydrazine in refluxing ethyl alcohol afforded the

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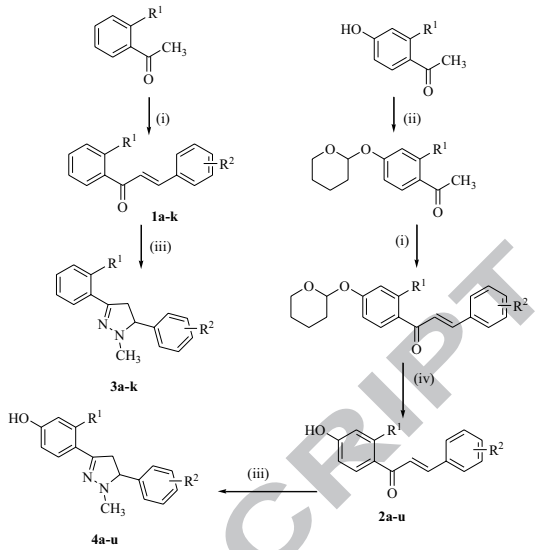
corresponding 4,5-dihydro-1*H*-pyrazoles **3a-k** and **4a-u** (see Supplementary material).

All synthesized pyrazolines were screened for their potential effects towards the two human MAO isoforms. (physicochemical data, and anti-MAO efficacies are reported in Table 1).

The inhibitory activities were investigated by measuring the effects of each derivative on the production of hydrogen peroxide (H_2O_2) from p-tyramine, using the Amplex® Red MAO assay kit (Molecular Probes, Inc., Eugene, Oregon, USA) and microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for human MAO-A or MAO-B (Sigma–Aldrich Química S.A., Alcobendas, Spain). The production of H_2O_2 catalysed by MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex® Red reagent), a non-fluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. In this study, MAO inhibition was evaluated using the above mentioned method, following the general procedure previously described by us.[20]



Scheme 1. Reagents and conditions: (i) substituted benzaldehyde, EtOH, $Ba(OH)_2$ x $8H_2O$, 30°C, 24h. (ii) 3,4-dihydro-2-*H*-pyran, CH_2Cl_2, 12h. (iii) methylhydrazine, KOH, EtOH, 80°C, 24h. (iv) 3N HCl , EtOH.

Table 1. Chemical data, physical data, and biological activity of 1-methyl-3,5-diphenyl-4,5-dihydro-1*H*-pyrazoles (**3a-k** and **4a-u**) and reference inhibitors.

Compd	R^1	R^2	m.p. (°C)	Purification method	Yield%	hMAO-A (IC_{50}) µM	hMAO-B (IC_{50}) µM	SI[c]
3a	H	H	Oil	A	63	1.68 ± 0.03[a]	0.12 ± 0.01	14
3b	H	2F	164-166	B	28	2.84 ± 0.02[a]	0.28 ± 0.03	10
3c	H	3F	Oil	A	41	1.82 ± 0.05[a]	0.06 ± 0.01	30
3d	H	4F	32-35	B	61	3.00 ± 0.06[a]	0.14 ± 0.01	21
3e	H	$2CH_3$	Oil	A	46	3.26 ± 0.08[a]	0.11 ± 0.01	30
3f	H	$3CH_3$	Oil	A	46	***	0.11 ± 0.01	> 909[#]
3g	H	$4CH_3$	30-33	B	42	1.39 ± 0.07[a]	0.0096 ± 0.0003	145
3h	H	2Cl	Oil	A	83	**	3.65 ± 0.29	> 27[#]
3j	H	3Cl	Oil	A	13	50.99 ± 1.33[a]	0.84 ± 0.06	61
3k	H	4Cl	40-44	B	40	**	3.95 ± 0.15	> 25[#]
4a	H	H	195-198	C	37	***	26.80 ± 1.95	> 3.7[#]
4b	H	2F	172-175	C	29	***	3.04 ± 0.17	> 33[#]
4c	H	3F	115-118	D	33	***	3.17 ± 0.21	> 31[#]
4d	H	4F	175-177	C	15	***	3.65 ± 0.16	> 27[#]
4e	H	$2CH_3$	105-107	C	29	21.87 ± 0.99	20.08 ± 1.01	1.1
4f	H	$3CH_3$	155-157	C	43	16.33 ± 0.83[b]	5.78 ± 0.27	2.8
4g	H	$4CH_3$	150-152	C	35	9.13 ± 0.52[a]	1.91 ± 0.06	4.8
4h	H	2Cl	128-131	B	20	11.82 ± 0.97	10.44 ± 0.02	1.1
4j	H	3Cl	130-133	C	40	18.69 ± 1.36[a]	2.07 ± 0.13	9
4k	H	4Cl	184-185	C	37	12.73 ± 0.8[a]	2.64 ± 0.19	4.8
4l	OH	H	136-139	B	36	***	***	-
4m	OH	2F	78-80	B	36	***	3.87 ± 0.27	> 26[#]
4n	OH	3F	123-126	B	20	**	**	-
4o	OH	4F	72-75	D	31	0.81 ± 0.04[b]	0.49 ± 0.03	1.6
4p	OH	$2CH_3$	66-69	D	36	3.98 ± 0.12[b]	2.04 ± 0.15	1.9
4q	OH	$3CH_3$	63-65	D	61	***	***	-
4r	OH	$4CH_3$	145-148	D	12	***	***	-
4s	OH	2Cl	55-56	D	25	1.10 ± 0.12 [a]	0.30 ± 0.02	3.7
4t	OH	3Cl	60-62	D	19	0.42 ± 0.06[b]	0.18 ± 0.02	2.3
4u	OH	4Cl	65-67	D	33	1.39 ± 0.09[a]	0.34 ± 0.03	4.1
Clorgyline						0.0045 ± 0.32 [a]	61.35 ± 1.13	0.000073
Selegiline						67.25 ± 1.02[a]	0.0196 ± 0.86	3362
Iproniazid						6.56± 0.76	7.54 ± 0.36	0.87
Moclobemide						361± 19.37	*	< 0.36

A= Chromatography on $SiO_2/CHCl_3$; B = crystallized from methanol; C = crystallized from acetonitrile; D = crystallized from n-hexane
All IC_{50} values shown in this table are the mean ± S.E.M. from five experiments. Level of statistical significance: [a]P < 0.01 or [b]P < 0.05 versus the corresponding IC_{50} values obtained against hMAO-B, as determined by ANOVA/Dunnett´s. ** Inactive at 100 µM (highest concentration tested). *** 100 µM inhibits the corresponding MAO activity by approximately 40-50%. At higher concentration the

compounds precipitate. [c]SI: hMAO-B selectivity index = IC_{50} (hMAO-A)/ IC_{50} (hMAO-B). # Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).

All the new synthesized compounds (**3a-k** and **4a-u)** and reference inhibitors were tested towards hMAO isoforms. Results reported in Table 1 show that most derivatives inhibited the hMAO-B isoform with IC_{50} values in the low micromolar or nanomolar range. Moreover, several compounds proved to be able to inhibit hMAO-A with lower affinity. Therefore, the new compounds are in general selective hMAO-B inhibitors.

All the compounds unsubstituted on the 3-phenyl ring (**3a-k**) were associated with potent and selective hMAO-B inihibitory activity. 1-Methyl-3-phenyl-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole **3g** was the most potent hMAO-B inhibitor identified in this study with an IC_{50} of 9.6 nM coupled with high selectivity (SI = 145). In comparison with the reference inhibitors, **3g** was at least 2-fold more active. The displacement of the *para* methyl group to the *meta* position on the 5-phenyl ring resulted in compound **3f**, approximately 11-fold less potent as a hMAO-B inhibitor than the corresponding *para* methyl analogue **3g**. However, **3f** exhibited the highest hMAO-B selectivity due to its poor hMAO-A affinity up to the highest concentration tested (100 μM).

Generally, the introduction of a hydroxyl group at the *para* position of the 3-phenyl ring (**4a-k**) led to a reduction of efficacy against both isoforms with respect to unsubstituted analogues **3a-k**. In fact, all the 3-(4-hydroxyphenyl)-1-methyl-5-phenyl-4,5-dihydro-1*H*-pyrazoles (**4a-k**) exhibited hMAO inhibition potency in the micromolar range. In this series of compounds, the presence of a fluorine substituent on the 3-phenyl ring led to selective hMAO-B inhibitors (**4b-d**), while the introduction of either a chlorine or a methyl group resulted in compounds **4e-k** endowed with low or modest hMAO-B selectivity.

Moreover, the introduction of a hydroxyl substituent at the *ortho* position of 3-phenyl ring, gave access to compounds **4l-u**, that showed different results. While compounds **4l, 4n, 4q** and **4r** are essentially inactive against both hMAO isoforms, **4o** and **4t** displayed potency in the submicromolar range against both hMAO-A and hMAO-B resulting in compounds endowed with poor selectivity. Moreover, the chloro-substituted derivatives **4s-u** exhibited enhanced inhibitory potency with respect to the corresponding 3-phenyl and 3-(4-hydroxyphenyl) analogues (**3h-k** and **4 h-k**, respectively).

By comparing the hMAO-B inhibitory activity of the new N1-methyl derivatives (**3a-k** and **4a-u**) with that of recently reported N1-thiocarbamoyl analogues,[16g,h] it is possible to point out that the introduction of a smaller group, such as the methyl one, led to compounds more active and selective towards hMAO-B isoform. Moreover, most of the previously studied 3,5-diphenyl-4,5-dihydro-1H-pyrazole derivatives substituted at N1 position of the pyrazoline ring with phenyl, acyl-, or thiocarbamoyl-groups were evaluated using rat or bovine instead of human MAOs. Since species-dependent differences in substrate specificity and inhibitor selectivity have been reported,[16a-c,e] no further comparison is possible.

We can conclude that in this series of inhibitors, an unsubstituted 3-phenyl ring seems to be an important chemical feature to obtain potent and selective hMAO-B inhibitors. Furthemore, the presence of the chlorine substituent at the 5-phenyl ring, coupled with the presence of two hydroxyl groups at both the 2' and 4' positions of the 3-phenyl ring, enhances both hMAO-A and hMAO-B inhibition efficacies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version,