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New 4-(4-methyl-phenyl)phthalazin-1(2*H*)-one derivatives and their effects on α_1-receptors

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Abstract—Continuing our research aimed at obtaining new compounds with high affinity and selectivity toward α_1-AR, a new series of arylpiperazine derivatives was designed, synthesized, and biologically tested. The new compounds **1**–**17** are characterized by a phenylphthalazin-1(2*H*)-one fragment connected through an alkyl chain to an arylpiperazine residue. The pharmacological profile of these compounds was evaluated for their affinity and selectivity toward α_1-AR, α_2-AR and toward $5HT_{1A}$ serotoninergic receptor. A discussion on the structure–activity relationship (SAR) of these compounds is also reported.

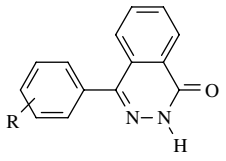
The α_1-adrenoceptors (α_1-AR) are a family of G-protein-coupled seven-transmembrane helix receptors comprising of multiple subtypes. Currently, three distinct α_1-adrenoceptors—α_{1A}, α_{1B}, and α_{1D}—have been defined in native tissues and their c-DNA has been cloned and expressed in various cell lines, α_{1a}, α_{1b}, and α_{1d}.[1]

α_1-Adrenoceptors are therapeutically interesting because of their involvement in the control of blood pressure[2,3] and for benign prostatic hyperplasia (BPH).[4]

The fact that α_1-blockers have been used in the treatment of benign prostatic hyperplasia (BPH) for more than two decades and combined with experimental data showing that BPH is the most common benign tumor in men has led in recent years to a marked increase in the search and development of new α_1-AR antagonists.[5]

It is well known that the addition of arylpiperazinylalkyl side chains into different heterocycles, such as uracils or the pyrimido[5,4-*b*]-indole moiety,[6] provides compounds that effectively lower blood pressure by antagonizing the α_1-AR. Among the compounds that show high affinity toward α_1-ARs, considerable attention has been given to molecules containing an arylpiperazinyl moiety as the pharmacophoric portion.

There are many reports in the literature regarding compounds containing a pyridazin-3(2*H*)-one fragment and their potential biological activities as antihypertensive agents; compounds GYKI-12743[7] or phenyl-phthalazinone **I**[8,9] are examples. This literature survey led to the suggestion that the arylpiperazinyl and pyridazinone moieties are key elements for α_1-AR affinity.



I R = H
R = $NHNH_2$

In the course of our studies in the field of new and selective α_1-AR ligands, containing a pyridazin-3(2*H*)-one ring, we have recently synthesized several compounds having an arylpiperazinylalkyl chain at the 2-position of the pyridazinone moiety, and a group such as furoyl-piperazine or 1,4-benzodioxan-2yl-methyl-piperazine at the 4-, 5- or 6-positions. Some of these compounds showed high affinity for α_1-ARs.[10–14]

Abbreviation: TBAB, tetrabutyl ammonium bromide.
Keywords: α_1-Adrenoceptor affinity; α_2-Adrenoceptor affinity; $5HT_{1A}$-serotoninergic affinity; Phenyl-phthalazinone fragment; Arylpiperazines, Structure–activity relationships (SAR).
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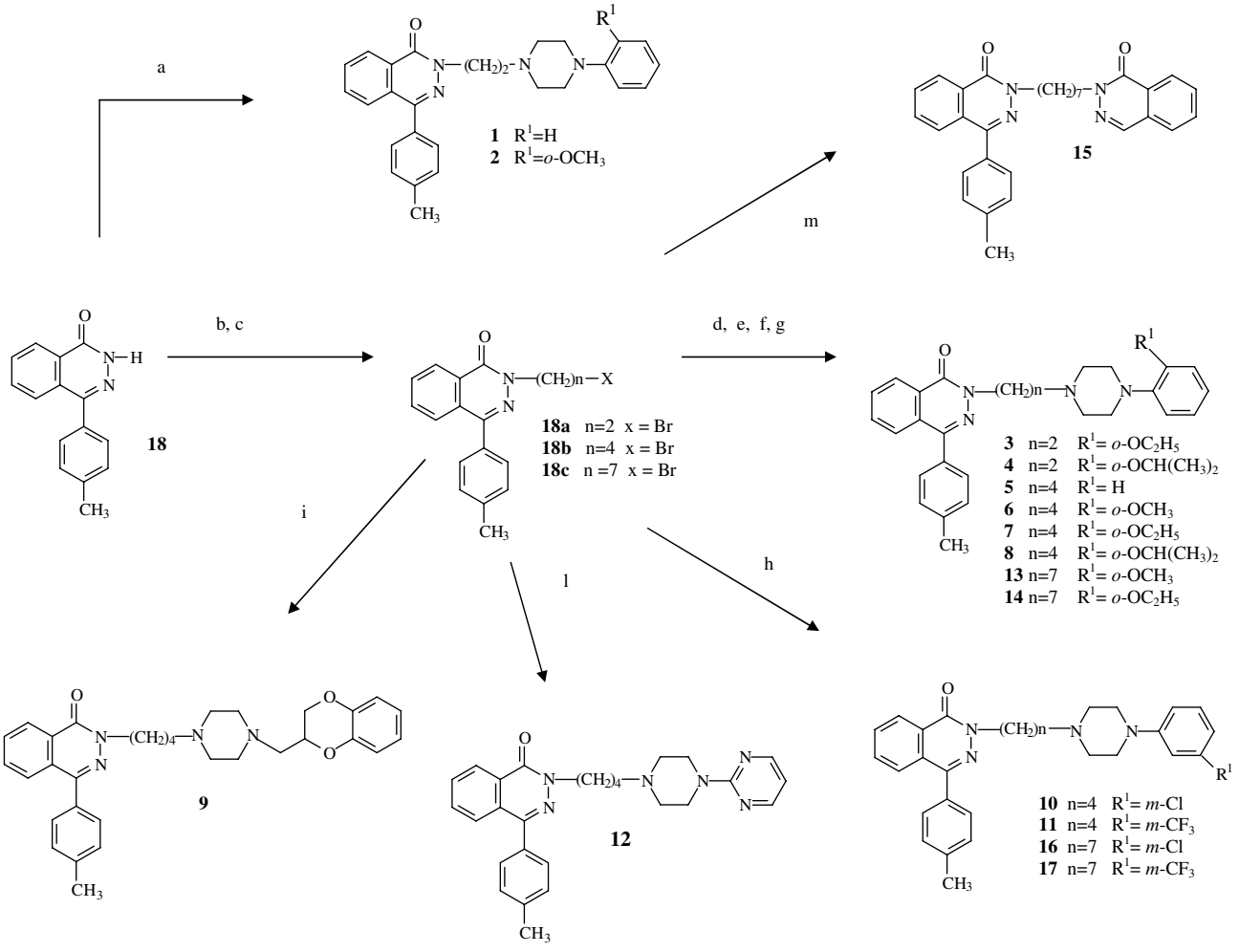
Based on the results from our previous works,[10–14] the new compounds that have an *ortho*-substituted phenylpiperazinylalkyl pyridazinone moiety as the common chemical scaffold, which allows for variations to be introduced on the terminal moiety linked to the pyridazinone nucleus and on the size of the *ortho*-alkoxy constituent, were designed and prepared for study.

Moreover it was reported by us,[11,12] that a gradual increase in affinity may be obtained by lengthening the polymethylene spacer between the pyridazinone and arylpiperazine moieties of the piperazinylalkylpyridazinone derivatives. On the other hand, α_2/α_1 selectivity is mainly dependent on the characteristics of the terminal molecular fragment directly linked to the pyridazinone ring. On the basis of these considerations and taking into account our previous experience in this field, our goal was to discover novel compounds characterized by good affinity for α_1-AR, possibly, high selectivity toward α_1-AR with respect to α_2-AR or to $5HT_{1A}$.

In this work, we report the synthesis and pharmacological data of new compounds in which the phenyl-phthalazin-1(2*H*)-one fragment is present as terminal molecular moiety.

The objective of this paper was to probe the importance of this phenyl-phthalazinonic fragment in relationship to the affinity and selectivity toward α_1-AR, when the arylpiperazinyl fragment is present and the alkyl chain as the spacer between the two major constituents of the molecule.

Compounds containing a 4-(4-methylphenyl)phthalazin-1(2*H*)-one fragment, connected to an arylpiperazine moiety by an alkyl chain of two, four or seven carbon



Scheme 1. Reagents: (a) acetone, dry potassium carbonate, 1-(2-chloroethyl)-4-phenylpiperazine (**19**), 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (**19a**); (b) $Br(CH_2)_2Br$, TBAB, KOH, benzene; (c) acetone, dry potassium carbonate, $Br(CH_2)_4Br$, or $Br(CH_2)_7Br$; (d) acetonitrile, dry potassium carbonate, 1-(2-ethoxyphenyl)piperazine (**20**); or 1-(2-isopropoxyphenyl)piperazine (**20a**); (e) acetonitrile, dry potassium carbonate, 1-phenylpiperazine (**20d**), or 1-(2-isopropoxyphenyl)piperazine (**20a**); (f) acetonitrile, dry potassium carbonate, 1-(2-methoxyphenyl)piperazine (**20e**), or 1-(2-ethoxyphenyl)piperazine (**20**); (g) isoamyl alcohol, dry potassium carbonate, 1-(2-methoxyphenyl)piperazine (**20e**), or 1-(2-ethoxyphenyl)piperazine (**20**); (h) acetonitrile, dry potassium carbonate, 1-(3-chlorophenyl)piperazine (**20b**) or 1-(3-trifluoromethylphenyl)piperazine (**20c**); (i) acetonitrile, dry potassium carbonate, 1-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-piperazine (**21**); (l) acetonitrile, dry potassium carbonate, 2-piperazin-1-yl-pyrimidine (**22**); (m) acetone, dry potassium carbonate, 3,4-dihydrophthlalazin-1(2*H*)-one (**23**).

atoms, were synthesized (compounds **1**–**8**, **10**–**14**, **16**, and **17**).

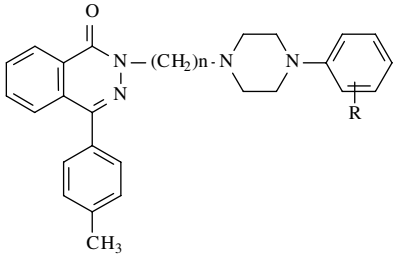
Moreover, to confirm the importance of the arylpiperazine fragment as the pharmacophoric portion with respect to the α_1-AR affinity, an arylpiperazine moiety was replaced by 1,4-benzodioxan-2-yl-methyl-piperazine or a phthlazin-1(2*H*)-one group (compounds **9** and **15**). The synthetic pathways to compounds **1**–**17** are shown in Scheme 1.

Alkylation of 4-*p*-tolyl-2*H*-phthalazin-1-one (**18**) with 1-(2-chloroethyl)-4-phenylpiperazine (**19**) or with 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (**19a**) (both synthesized following the method described by Bourdais)[15] in acetone in the presence of dry potassium

Table 1. Chemical and physical data of compounds **1**–**17**

Compound	Formula	Mp (°C)	Yield (%)
1	$C_{27}H_{28}N_4O{\cdot}2HCl{\cdot}1/2H_2O$	190–191	20
2	$C_{28}H_{30}N_4O_2{\cdot}2HCl{\cdot}1/2H_2O$	224–226	40
3	$C_{29}H_{32}N_4O_2{\cdot}2HCl{\cdot}H_2O$	218–220	63
4	$C_{30}H_{34}N_4O_2{\cdot}2HCl$	196–198	80
5	$C_{29}H_{32}N_4O{\cdot}2HCl$	208–212	90
6	$C_{30}H_{34}N_4O_2{\cdot}2HCl{\cdot}1/2H_2O$	204–206	70
7	$C_{31}H_{36}N_4O_2{\cdot}2HCl$	199–200	50
8	$C_{32}H_{38}N_4O_2{\cdot}2HCl{\cdot}H_2O$	177–179	80
9	$C_{32}H_{36}N_4O_3{\cdot}2HCl{\cdot}H_2O$	240–244	60
10	$C_{29}H_{31}ClN_4O{\cdot}HCl{\cdot}1/2H_2O$	205–207	45
11	$C_{30}H_{31}F_3N_4O{\cdot}2HCl$	156–158	70
12	$C_{27}H_{30}N_6O{\cdot}2HCl{\cdot}1/2H_2O$	227–229	80
13	$C_{33}H_{40}N_4O_2{\cdot}3HCl$	162–168	40
14	$C_{34}H_{42}N_4O_2{\cdot}1HCl$	155–157	45
15	$C_{30}H_{30}N_4O_2.HCl$	40–44	30
16	$C_{32}H_{37}N_4OCl{\cdot}HCl{\cdot}1/2H_2O$	96–100	45
17	$C_{33}H_{37}F_3N_4O{\cdot}HCl$	170–172	60

Table 2. α_1-AR, α_2-AR, and $5HT_{1A}$ serotoninergic receptor binding affinities of compounds **1**–**8**, **10**, **11**, **13**, **14**, **16**, and **17**



Compound	*n*	R	K_i[a] (nM)				
			α_1-AR	α_2-AR	5-HT_{1A}	Ratio α_2/α_1	Ratio $5HT_{1A}/\alpha_1$
1	2	H	25.3 ± 0.12	4262 ± 1469	6200 ± 870	168.5	245.0
2	2	*o*-OCH_3	3.5 ± 1.48	578 ± 269.3	473 ± 105	165.0	135.1
3	2	*o*-OC_2H_5	3.9 ± 0.82	867 ± 95	951 ± 100	222.3	244.0
4	2	*o*-$OCH(CH_3)_2$	5.0 ± 0.70	1301 ± 140	1288 ± 182	260.2	257.6
5	4	H	23.9 ± 5.11	186 ± 36	450 ± 95	7.8	18.8
6	4	*o*-OCH_3	2.6 ± 0.51	60.37 ± 10	38.5 ± 4.7	23.2	14.8
7	4	*o*-OC_2H_5	2.23 ± 0.48	60.47 ± 15	41 ± 7.5	27.1	18.3
8	4	*o*-$OCH(CH_3)_2$	1.07 ± 0.18	96 ± 32	81 ± 35	89.7	75.7
10	4	*m*-Cl	12.4 ± 4.07	178.2 ± 20	116 ± 11	14.4	9.3
11	4	*m*-CF_3	111.6 ± 11	186.5 ± 16.2	71.8 ± 14	1.6	1.5[b]
13	7	*o*-OCH_3	8.4 ± 0.6	105 ± 10.5	101 ± 28	12.5	12.0
14	7	*o*-OC_2H_5	7.33 ± 1.2	889 ± 160	207 ± 35	121.3	28.2
16	7	*m*-Cl	37.0 ± 7.0	430.0 ± 43.0	673.0 ± 75.0	11.6	18.2
17	7	*m*-CF_3	382.0 ± 70	4180 ± 1000	722 ± 160	10.9	1.9
P			0.24 ± 0.05		>10,000		
R				4.0 ± 0.3			
D					2.0 ± 0.2		

[a] The K_i values are means ± SD of a series of separate assays, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff:[20] $K_i = IC_{50}/1 + (L/K_d)$, where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin (**P**) binding to rat cortex membranes was 0.24 nM (α_1), K_d of [^{3}H]rauwolscine (**R**) binding to rat cortex membranes was 4 nM (α_2), and K_d of [^{3}H]8-OH-DPAT (**D**) binding to rat cortex membranes was 2 nM ($5\text{-}HT_{1A}$).

[b] $\alpha_1/5HT_{1A}$ ratio.

carbonate, at reflux, afforded compounds **1** and **2**, respectively, in moderate yield.

Starting with **18**, intermediate **18a** was obtained, by alkylation with 1,2-dibromoethane under phase transfer catalysis, according to the procedure reported by Yamada.[16] In turn, **18a** was converted to the final compounds **3** and **4**, in 60–80% overall yield, by reacting it with 1-(2-ethoxyphenyl)piperazine (**20**) or 1-(2-isopropoxyphenyl)piperazine (**20a**),[17] respectively, in acetonitrile/dry potassium carbonate at reflux.

The alkylation of **18** with α,ω-dibromoalkanes having four or seven methylene groups was used to prepare intermediates **18b** and **18c**, respectively (dry potassium carbonate/acetone, at reflux). Compounds **5** and **8** were prepared by reacting intermediate **18b** with 1-phenylpiperazine (**20d**) or 1-(2-isopropoxyphenyl)piperazine (**20a**), respectively, in acetonitrile in the presence of dry potassium carbonate at reflux. Using the same procedure as that described for compound **5**, compounds **13** and **14** were obtained by reacting intermediate **18c** with 1-(2-methoxyphenyl)piperazine (**20e**) or 1-(2-ethoxyphenyl)piperazine (**20**), respectively.

Compounds **6** and **7** containing a fragment of 1-(2-methoxyphenyl)piperazine or 1-(2-ethoxyphenyl)piperazine, respectively, were prepared by using isoamyl alcohol and dry potassium carbonate at reflux.

Compounds **10** and **11**, **16** and **17** containing a linker of four or seven carbon atoms, respectively, were prepared by direct alkylation of **18b** or **18c** with the 1-(3-chlorophenyl)piperazine (**20b**) or 1-(3-trifluoromethylphenyl)-piperazine (**20c**), according to the procedure reported for the synthesis of compound **5**.

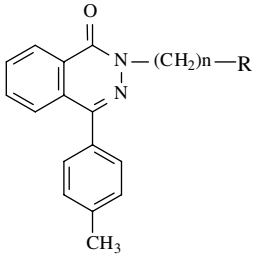
Compounds **9** and **12**, were prepared by alkylation of **18b**, with 1-(2,3-dihydrobenzo[1,4]dioxin-2-ylmethyl)-piperazine (**21**) or with 2-piperazin-1-yl-pyrimidine (**22**), respectively, using the same procedure described above for compound **5**.

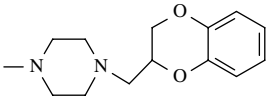
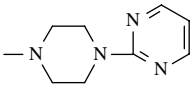
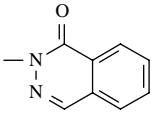
Finally, compound **15** was prepared, starting with the intermediate **18c** using 3,4-dihydrophthalazin-1(2*H*)-one (**23**), acetone/dry potassium carbonate, as described above for compound **5**.

The chemical and physical data for these compounds are reported in Table 1.[18]

The pharmacological profiles of these compounds were evaluated for their affinities toward α_1-AR, α_2-AR, and 5-HT_{1A} serotoninergic receptor by determining the ability of each compound to displace [^{3}H]prazosin, [^{3}H]rauwolscine, and [^{3}H]8-OH-DPAT, respectively, from specific binding sites on rat cerebral cortex.[19] K_i values were determined on the basis of three competition binding experiments in which seven drug concentrations, run in triplicate, were used.[20]

Table 3. α_1-AR, α_2-AR, and $5HT_{1A}$ serotoninergic receptor binding affinities of compounds **9**, **12**, and **15**



Compound	*n*	R	K_i[a] (nM)				
			α_1-AR	α_2-AR	5-HT_{1A}	Ratio α_2/α_1	Ratio $5HT_{1A}/\alpha_1$
9	4		180.0 ± 30	278.0 ± 48	1928.0 ± 340	1.5	10.7
12	4		270.0 ± 19.5	2034 ± 150	1181 ± 170	7.5	4.4
15	7		26%[b]	15%[b]	10%[b]	nd	nd
P			0.24 ± 0.05		>10,000		
R				4.0 ± 0.3			
D					2.0 ± 0.2		

[a] The K_i values are means ± SD of series separate assays, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff:[20] $K_i = IC_{50}/1 + (L/K_d)$, where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]prazosin (**P**) binding to rat cortex membranes was 0.24 nM (α_1), K_d of [^{3}H]rauwolscine (**R**) binding to rat cortex membranes was 4 nM (α_2), and K_d of [^{3}H]8-OH-DPAT (**D**) binding to rat cortex membranes was 2 nM (5-HT_{1A}).

[b] Percent inhibition at the 10 μM dose.

The pharmacological data reported in Tables 2 and 3 confirm that:

The phenylpiperazine system is essential for determining affinity toward all the receptors considered. In fact, the compounds bearing a 2-methyl-2,3-dihydrobenzo[1,4]dioxine (compound **9**) or a phthalazinonic nucleus (compound **15**) were characterized by a significant decrease in affinity toward the previously described receptor. This could be due to differences in the hydrophilic/lipophilic nature of this portion of the molecule, as suggested by our previous studies.[10–14] In addition, the literature reports that there is usually a decrease in affinity toward α_1-AR with simple heterocyclic rings, such as pyrimidine (compound **12**).

It is confirmed that increasing the bulkiness of the alkoxy group at the *ortho*-position of the phenyl ring attached to the piperazine nucleus leads to an enhanced affinity toward α_1-AR. Some examples are compounds **1**–**8**; compound **8**, which has an isopropoxy group and a linker of four carbon atoms, showed the highest affinity. Moreover, it has been confirmed that, a *meta*-substitution leads to a marked drop in affinity toward α_1-AR. Compounds **10**, **11**, **16**, and **17** in which a chlorine atom or CF_3 group is present, the affinity values toward α_1-AR are several orders of magnitude higher than those of the *ortho*-substituted compounds (compounds **6**, **7**, **13**, and **14**).

The different ethyl-, butyl- or heptyl-chain extensions that constitute the spacers between the arylpiperazine system and the terminal heterocyclic fragment exerted a weak influence determining selectivity of the new compounds toward α_1-AR. Compounds **1**–**4** containing a two-carbon atom linker showed the better selectivity; compound **4**, with an *o*-isopropoxy group, showed the highest selectivity toward the α_1-AR (α_2/α_1 = 260 or $5HT_{1A}/\alpha_1$ = 257). In contrast, lengthening the spacer to a four, or seven methylene unit, decreased the selectivity toward α_1-AR; compounds **5**–**8**, **7**, **13**, and **14** are examples.

In conclusion, for these compounds, in which is present 4-*p*-tolyl-2*H*-phthalazin-1-one such as terminal molecular fragment, a four-carbon atom spacer is the best polymethylene chain for affinity; while a two-carbon atom spacer is the best for selectivity. Therefore, the selectivity toward the α_1-AR receptor can be improved, by blocking the terminal heterocyclic moiety linked to pyridazinone nucleus and by shortening the chain that connects the two parts of the molecule.

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