# Allosteric Modulation of Dopamine Receptors

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**Abstract:** Allosteric modulators allow for the fine-tuning of receptor responses to endogenous neurotransmitters and exogenous therapeutic agents. Different types of allosteric modulation of dopamine receptors are discussed as well as the significance of such modulation in the control of normal biological processes and in the treatment of disease.

**Keywords:** Conformational change, regulation, modulate, plateau, catecholamine, biogenic amine, G protein-coupled receptor (GPCR), metal.

## INTRODUCTION

Allosteric modulators are agents that remotely alter the interactions of ligands with their receptors by modifying the ligand-binding environment. An example of this type of modulation is when the binding of a modulator to an allosteric (secondary) site produces a conformational change in the receptor protein that is transmitted to the ligand's orthosteric (primary) binding site. The quality of the allosteric effect is said to be positive if the modulator facilitates an interaction or negative if it inhibits an interaction of the ligand with the orthosteric binding site. The degree of coupling between the allosteric site and orthosteric site, or cooperativity, can be high or low. If an allosteric effect is highly cooperative then the concentration dependent effect of the modulator on the ligand's affinity will plateau only at high concentrations. Noncompetitive interactions, which result in a complete occlusion of the ligand binding site leading only to a decrease in receptor density (and no change in ligand affinity), are also allosteric in nature but they are a special case of neutral cooperativity and are concentration-independent effects. At dopamine receptors, all three modes of allosteric modulation occur: positive, negative and neutral cooperativity. For instance, the binding of sodium ions to dopamine receptors reduces the receptors' affinity for agonists by converting receptors that are in the high affinity state to the low affinity state ($R_H \rightarrow R_L$). In contrast, the tripeptide proline-leucine-glycine (PLG) potentiates agonist binding to dopamine receptors both by converting receptors in the low affinity state to the high affinity state ($R_L \rightarrow R_H$) and by increasing the affinity of the high affinity state. Amiloride, and its nitrogen substituted analogues, such as methylisobutylamiloride (MIA), allosterically inhibit the binding of antagonists to all dopamine receptor subtypes. Even though the macroscopic outcome (e.g., inhibition) is similar for all the subtypes, the degree of cooperativity and the precise molecular mechanisms are subtype-specific. Like MIA, zinc has been shown to be a negative heterotropic allosteric modulator of antagonist binding to certain dopamine receptors subtypes, while at other subtypes it exerts a noncompetitive allosteric effect. Still other allosteric modulators, like SCH202676, appear to be solely noncompetitive allosteric modulators of antagonist binding to dopamine receptors. Thus, there exists a diverse range of allosteric mechanisms (sites) for modulating the effects of endogenous and therapeutic agents that target dopamine receptors.

The ability of allosteric modulators to fine tune pharmacological responses has sparked interest in their potential applications in both clinical and basic science settings. For example, when PLG is utilized as an adjuvant with L-DOPA it potentiates behaviors indicative of an anti-parkinsonian drug effect over that seen with L-DOPA alone, i.e., increased contralateral rotations in 6-hydroxydopamine hemilesioned rats. The idea is that a PLG-like adjuvant might be employed to lower the therapeutic threshold for L-DOPA [1,2], which should extend effective treatment periods and reduce severe side effects [3-9]. An attractive safety feature of positively (and negatively) cooperative allosteric modulators in therapeutic applications is that the modulator's concentration-effect plateaus, which permits a broad therapeutic dose range and safe-guards against overdose. A knowledge of endogenous allosteric modulators like zinc and its ability to prevent antagonist binding to D2 dopamine receptors may also aid in the development of new therapies for those schizophrenics that do not respond to antipsychotic medications [10]. Certain allosteric modulators have been shown to display receptor subtype selectivity in terms of their rank order binding affinities and/or their molecular mechanisms of action, which might also be exploited in a clinical application. For example, an allosteric modulator that exerts a heterotropic neutral cooperativity would effectively reduce receptor density and a drug that had acted as a weak partial agonist at high receptor density would now function as a pure antagonist at lower receptor density. Because allosteric modulators are exquisitively sensitive probes of protein conformation, they have been utilized to determine whether a particular mutation produces global changes in protein conformation [11]. Understanding the molecular mechanisms of allosteric modulation may also provide some insight into the types of conformational states/transitions that might be occurring in the normal course of receptor functioning.

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## ALLOSTERIC MODULATION OF DOPAMINE RECEPTORS BY HYDROGEN AND SODIUM IONS

The ability of sodium ions to regulate the binding of agonists to biogenic amine (and other types of heterotrimeric) G protein-coupled receptors (GPCR) is perhaps the best known allosteric effect. Like high concentrations of GTP and slowly hydrolyzable GTP analogues, sodium ions, at millimolar concentrations, convert dopamine receptors from their high affinity state ($R_H$) to their low affinity state ($R_L$), which decreases agonist binding. Although the binding of most antagonists is sodium-resistant, the binding of many substituted benzamide antagonists to dopamine receptors is enhanced by sodium ions. Wide ranges of magnitudes of the potentiating effect of sodium on the binding of various different substituted benzamide antagonists have been reported (2-fold to about 40-fold) [12-14]. This enhancement of substituted benzamide antagonist binding is most often observed as an increase in binding affinity; however, increases in Bmax with no change in affinity have also been observed for certain less sodium-sensitive substituted benzamides and may be due to differences in binding conditions (e.g., differences in wash buffer conditions). For example, the two-fold, sodium-dependent increase in [$^3$H]raclopride binding to cloned D2 dopamine receptors expressed in CHO cells has been reported to be purely an effect on affinity or Bmax [10, 14].

The molecular site of sodium interaction with dopamine receptors, like that of other biogenic amine and certain peptide GPCRs [15], has been shown to rely upon a conserved aspartic acid residue at position 2.50 (D2.50) [13, 16, 17]. The importance of a negatively-charged carboxyl group at position 2.50 in the electrostatic attraction of positively-charged sodium ions has been established by mutational studies of D2 and D4 dopamine receptors. For example, the sodium-sensitivity of both dopamine and zinc binding to the D4 dopamine receptor is selectively abolished by the charge-neutralizing D2.50N mutation, even though dopamine, zinc and [$^3$H]methylspiperone still bind the D2.50N mutant with near wild type affinities [18]. In addition, the sodium-sensitivity of agonist and substituted benzamide antagonist binding to the D2 dopamine receptor is abolished in the D2.50A mutant, but partly spared in the D2.50E mutant [16]. Even though dopamine still binds to these mutant D2.50A and D2.50E receptors, their ability to stimulate dopamine-induced coupling to G proteins is either abolished or severely diminished relative to the wild type receptor. All these results suggest that D2.50 is a critical site for the action of sodium on dopamine receptors and that the integrity of the (unoccupied) sodium site is somehow essential for maintaining a receptor conformation that is able to couple with G proteins. More recent studies suggest that amino acids other than D2.50 either may influence or form part of the site for sodium binding to D2 dopamine receptors. For example, it has been suggested in a revised model that the sodium binding pocket is shaped like a pyramid in three-dimensional space with D2.50, S3.39, N7.45 and S7.46 forming the base of the pyramid and the carbonyl backbone of N7.45 forming the apex, and sodium occupying the center [19]. Charge-neutralizing mutations at S3.39 or S7.46 resulted in diminished sensitivity to sodium for substituted benzamide antagonists, which lend some support for this revised model of the sodium binding site.

Like sodium, low pH or conditions that increase the concentration of hydrogen ions weakens agonist binding [13], and in fact, sodium and hydrogen ions appear to share a common allosteric binding site (D2.50) [16]. That hydrogen and sodium ions share a common binding site is perhaps not too surprising when one considers that hydrogen ions are often grouped as a 1A metal in the periodic table. In addition, other 1A metals like potassium and lithium have diminished interactions at the sodium site on dopamine receptors [12, 20] (and α2-adrenergic receptors [21]) as would be predicted by the Eisenman's cation selectivity principle if the sodium site has a strong field strength (i.e., sequence X) [22, 23].

Mutagenesis and modeling studies of D2 dopamine receptors [19] (and *in vitro* whole cell binding and functional assays on α2-adrenergic receptors [24]) suggest that the sodium binding pocket is only accessible from the intracellular side. High concentrations of sodium ions (>50 mM) are known to strongly promote the agonist low affinity state of dopamine receptors. This results in a decrease in agonist affinity and the subsequent uncoupling of the receptor from G proteins. The concentration of sodium inside intact resting mammalian cells is approximately 10 mM. Since the electrochemical gradient for sodium is directed toward the intracellular side, a possible physiological function of the sodium site that might explain its ability to allosterically modulate certain GPCRs may be that it acts as a combined activity sensor and cut-off switch that couples increased (sodium-permeable) channel activity with an attenuation of GPCR responsiveness. Such localized coupling between channels and heterotrimeric GPCRs may be relevant to a variety of disease states that are characterized by alterations in intracellular sodium, such as hypertension [24, 25], hyperthyroidism [26-28] and obesity [29, 30], and this coupling also may be an important aspect of the action of certain drugs, such as cardiac glycosides [31, 32] and glucocorticoids [33-36].

## ALLOSTERIC MODULATION OF DOPAMINE RECEPTORS BY ZINC IONS

Like sodium, zinc is a metal cation that allosterically modulates dopamine receptors. However, zinc is divalent rather than monovalent and its electronic configuration is very unlike that of sodium (a *d*-orbital versus an *s*-orbital), which in part accounts for the different rank-order preferences of sodium and zinc for their interactions with specific chemical moieties [23]. Furthermore, zinc inhibits the binding of both sodium-sensitive and sodium-insensitive antagonists to D2 dopamine receptors. In the case of the sodium-sensitive substituted benzamide antagonists, the molecular mechanisms of allosteric modulation by zinc are distinct from those of sodium [16, 10]. Not surprisingly then, zinc and sodium have been shown to occupy distinct allosteric sites on the D4 dopamine receptor [18]. For example, a mutant D4 dopamine receptor rendered sodium-insensitive by substitution of the negatively-charged aspartic acid at position 2.50 for a neutral asparagine (D2.50N) still binds zinc with wild type affinity.

Zinc ions moderately inhibit the binding of the agonist [$^3$H]propylnorapomorphine (NPA) to D2 dopamine receptors (unpublished results), but they robustly inhibit antagonist

binding. Although zinc allosterically modulates antagonist binding to all five dopamine receptors subtypes, its does so with distinct molecular mechanisms, and different affinities and magnitudes of cooperativity [37, 10]. For instance, zinc's negative heterotropic modulation is highly cooperative at D1 receptors, but weakly cooperative at D2 receptors (approximately a 10-fold difference in cooperativity). In contrast, zinc exerts neutral cooperativity at D4 receptors and it significantly accelerates one of the two dissociation rates for [$^3$H]methylspiperone at D3 receptors. Utilizing null pharmacological methods [38], the estimated equilibrium affinity constant ($K_D$) for zinc at the 'unoccupied' receptor (i.e., no [$^3$H]antagonist bound) is 9 μM at the D1 receptor and 40 μM at the D2 receptor [37]. Null methods have been employed to determine the affinity of zinc for its allosteric site on D1 and D2 receptors, because the apparent affinity for zinc varies in a manner that is dependent upon the concentration of antagonist present due to the reciprocally cooperative nature of zinc binding to the [$^3$H]antagonist occupied receptor. An unresolved issue is why in whole cell assays of functional antagonism of cloned D2 receptors zinc decreases the maximal reversal response by antagonist without significantly altering antagonist potency, when its primary effect on cloned D2 receptors in purified membranes from the same cells is on antagonist affinity [10].

Although the potential physiological relevance of zinc's modulation of D2-like antagonist binding is not clear, it may be therapeutically relevant in the case of antipsychotic drug treatments [10]. Approximately 15-20% of schizophrenics do not respond to antipsychotic drug treatments [39, 40]. Histological studies have revealed that high concentrations of zinc are stored in synaptic vesicles in several brain regions believed to be associated with schizophrenia [41-44]. Studies of depolarization-induced release of zinc from hippocampal neurons have yielded estimates of synaptic zinc levels of about 300 μM. Since zinc inhibits antagonist binding to the D2 dopamine receptor, and there is a strong correlation between the clinically efficacious dose of antipsychotic drugs and the affinity at which they antagonize the D2 dopamine receptor [45, 46], it is interesting to speculate that schizophrenics that are refractory to treatment with antipsychotic drugs might have elevated levels of synaptic zinc.

## ALLOSTERIC MODULATION OF DOPAMINE RECEPTORS BY AMILORIDE AND ITS NITROGEN-SUBSTITUTED DERIVATIVES

Amiloride (3,5-Diamino-N-(aminoiminomethyl)-6-chloropyrazinecarboxamide) (**1**) and its 5-amino- and guanidine-substituted derivatives Fig. (**1**) such as methylisobutylamiloride (**2,** MIA) and benzamil (**3**) inhibit antagonist binding to cloned rat dopamine D2L receptors [47]. The proposed mechanism is a mixed competitive/allosteric interaction, whereby MIA competes with [$^3$H]spiperone for the primary site and simultaneously facilitates its own binding to the competitive site *via* its binding to an allosteric site. Amiloride, benzamil and MIA all decrease [$^3$H]antagonist binding to cloned human D1 and D2-like (D2, D3, D4) dopamine receptors by accelerating [$^3$H]antagonist dissociation rates [48]. The rank order potency (and maximal increase) for this effect on antagonist dissociation rates is MIA > benzamil > amiloride, and MIA displays a greater than ten-fold selectivity for this effect on the D3 receptor subtype ($EC_{50}$ = 0.3 μM). The molecular mechanisms of this inhibition of [$^3$H]antagonist binding to cloned human D1, D2 and D3 dopamine receptors is best modeled as a mixed competitive/allosteric interaction (i.e., at two sites: the orthosteric site and an allosteric site) [48]. No remarkable differences have been observed between MIA binding interactions at D2L and D2S dopamine receptor splice variants. The molecular mechanisms for the inhibition of [$^3$H]spiperone binding to D4 dopamine receptors by benzamil and amiloride are best described by a purely competitive model. The precise mode of MIA's inhibition of [$^3$H]spiperone binding to D4 dopamine receptors could not be unambiguously assigned[48]. However, MIA binding to D4 dopamine receptors clearly has a strong allosteric component, because it accelerates [$^3$H]antagonist dissociation rates, and it has a pseudo Hill slope significantly less than unity in [$^3$H]spiperone/MIA pseudocompetition experiments. MIA also reduces the potency of dopamine-stimulated increases in [$^{35}$S]GTPγS binding mediated by the D2 dopamine receptor with no change in efficacy [48].

Amiloride is utilized in clinical applications as a weak potassium-sparing diuretic that promotes the excretion of water and sodium by the kidney. It is often used in combination with stronger, non-potassium sparing diuretics (e.g., hydrochlorothiazide and furosemide) in the treatment of hypertension [49, 50], nepherous diabetes insipidous [51, 52], and in cases of hypercalciuria [53, 54], because it prevents hypokalaemia. Amiloride exerts its therapeutic effect as a potassium-sparing diuretic by blocking amiloride-sensitive (and TTX-insensitive) sodium channels located in epithelial cells of the kidney [55]. Amiloride and certain of its derivatives are known to also interact with a wide variety of molecular targets such as ion channels, ion pumps, and GPCRs. For example, amiloride interacts with the apamin-sensitive and calcium-dependent small conductance potassium channels [56], T-type and L-type calcium

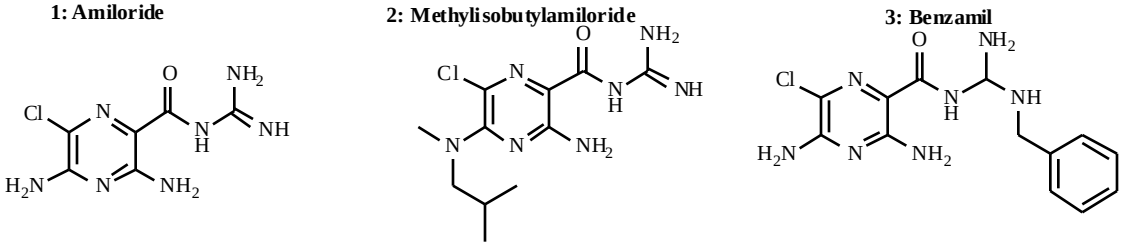



**Fig. (1).** Chemical structures of amiloride and a 5-pyrazinoyl-nitrogen derivative methylisobutylamiloride and a guanidine-nitrogen-substituted derivative benzamil.

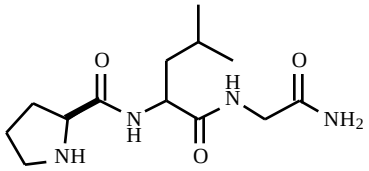
channels [57], the sodium-hydrogen exchanger [58], the sodium-calcium exchanger [59], and the sodium-potassium ATPase [60], as well as α2A and α2B adrenergic receptors [61-63], D1-D4 dopamine receptors [48] and A1, A2A and A3 adenosine receptors [64, 65]. While the rank order potencies of these different systems can be modified by preferentially derivatizing either the 5'-amino or 3'-guanido positions, the large number of known targets for amiloride would appear to make it a challenging pharmacophore on which to base the development of highly-subtype selective drugs.

## ALLOSTERIC MODULATION OF DOPAMINE RECEPTORS BY THE TRIPEPTIDE PROLINE-LEUCINE-GLYCINE (PLG) AND ITS PEPTIDE, SEMIPEPTIDE AND NONPEPTIDE MIMETICS

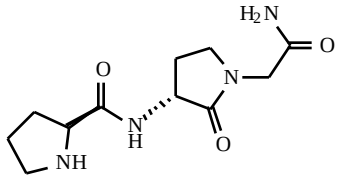
The tripeptide L-proline-L-leucine-L-glycine (**4,** PLG) Fig. (**2**) is a endogenous hypothalamic factor that inhibits the release of melanocyte stimulating hormone (MSH) from the anterior pituitary, and consequently, it is also know in the older literature as MSH release-inhibiting factor (MIF) or sometimes as MSH release-inhibiting hormone (MRIH) [66]. PLG potentiates the binding of the isoquinoline dopamine receptor agonist [$^3$H]apomorphine to D2 dopamine receptors in bovine striatal membranes [67] and rat striatal and hypothalamic membranes [68], while having no effecton the binding of the radiolabeled butyrophenone antagonist [$^3$H]spiperone. The potentiating effect on agonist binding is due to both an increase in the affinity of the high affinity state of the receptor and a GTP-insensitive increase in the proportion of receptors in the high affinity state [69]. However, PLG is unable to prevent a GTP-dependent change in the agonist affinity state when receptors are pre-incubated with a non-hydrolyzable GTP analogue Gpp(NH)p. The ability of PLG to increases the proportion of receptors in the high-affinity state correlates with its ability to increase the rate of propylnorapomorphine-induced GTPase activity in rat striatal tissues, and thereby, increases the amount of the dopamine receptor-Giα-GDPβγ complex corresponding to the high affinity state of the receptor [70]. A remarkable feature of PLG's actions is its asymmetrical parabolic dose-response curve – the potentiating effect of PLG increases with increasing concentration up to a maximum of around 1 μM and then slowly declines at higher concentrations [69]. PLG has a similar effect on another isoquinoline agonist (e.g., NPA, and an aminotetralin, e.g., 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN)) [69, 71].

A considerable amount of effort has been applied to understanding the structure-activity relationships (SAR) between PLG and potentiation of agonist interactions with dopamine receptors. Systematic substitution of each amino acid in PLG has revealed the following. Many substituents (i.e., D-proline, L-pyroglutamyl, L-piperdine, L-azetidine and L-3,4-dehydropropyl) can replace the proline with little effect on activity [72], while only a few substituents produce detrimental effects (i.e., D-3,4-dehydropropyl and L-thiazolidine). In contrast, fewer changes to the leucyl residue are well tolerated and those that are tolerated have narrow structural requirements [73]. For example, L-phenylalanine ($CH_2$-Phe) is an acceptable substitution for L-leucine as PFG has full activity, but either decreasing (e.g. Phe) or

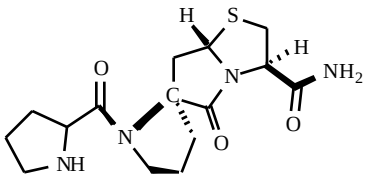
**4: D-Proline-D-Leucine-D-glycinamide (PLG)**



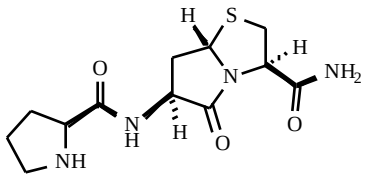
**5: PAOPA - conformationally constrained PLG peptidomimetic with a γ-lactam**



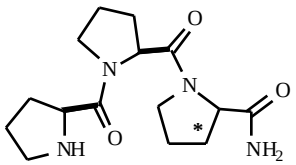
**6: Highly rigid and conformationally constrained PLG peptidomimetic with spiro 5,5-bicyclic thiazolidine lactam**



**7: Conformationally constrained PLG peptidomimetic with 5,5-bicyclic thiazolidine lactam**



**8: Proline-proline -prolylamide**



**9: PLG peptidomimetic with diketone piperazine substituting for proline**

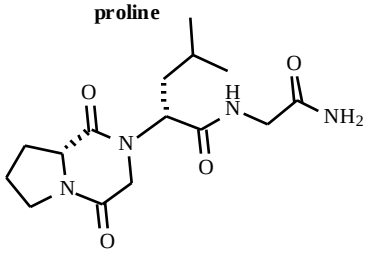


**Fig. (2).** Chemical Structures of PLG and its mimetics.

increasing the carbon spacer length (e.g., $CH_2$-$CH_2$-Phe) abolishes activity. Replacing L-leucine with a butyl moiety reduces maximal activity by about one-half, but increases the potency by approximately 10-fold. Like for the aromatic substitutions mentioned above, carbon chain length (and chain branching in this case) of the alkyl substitution is critical for maintaining activity. Substitution of the glycine residue with a variety of cyclic azosubstituents is well tolerated and some, such as (±)-thiazolidine-2-carboxamide and L-dehydroprolinamide, can significantly enhance the potentiating activity (2-3-fold) relative to the parent compound PLG [71].

The extraordinary sensitivity of leucine substitutions and their ability to increase potency lead to further investigations of the structural and conformational requirements at this central position. Initial studies revealed that a conformationally constrained γ-lactam analogue of PLG, 3(R)-(N-L-prolylamino)-2-oxo-1-pyrrolidineacetamide (**5,** PAOPA) Fig. (**2**), retains activity while drastically increasing potency (~1000-fold), but unlike PLG it must be pre-incubated with receptors in order to potentiate [$^3$H]ADTN binding [74]. Further studies have demonstrated the need for the PLG and its active derivatives to be able to adopt a type II β-turn conformation, in order to be active. For example, a highly rigid spiro 5,5-bicyclic thiazolidine lactam PLG derivative (**6**) is a type II β-turn mimetic [75] Fig. (**2**) that not only retains but has enhanced activity like the less rigid conformationally constrained 5,5-bicyclic (**7**) Fig. (**2**) and 6,5-bicyclic thiazolidine lactam derivatives, which are both capable of assuming a type II β-turn conformation [76]. In contrast, a stereoisomer of the most active 5,5-bicyclic thiazolidine lactam derivative that cannot adopt a type II β-turn conformation is totally inactive. That a type II β-turn is indeed the pharmacophore related to activity was elegantly demonstrated by showing that a tripeptide composed entirely of L-prolines (**8,** PPP) Fig. (**2**) that can adopt a type II β-turn conformation has comparable activity but even greater potency (~10-fold) than PLG [77].

In addition to both increasing the affinity of the agonist high affinity state and increasing the proportion of dopamine receptors in the high affinity state in *in vitro* radioligand binding studies, PLG and certain of its peptidomimetic derivatives have demonstrable activity in a variety of *in vivo* assays of dopamine receptor function. For example, chronic co-administration of PLG significantly attenuates haloperidol-induced catalepsy in rats [67, 78], and both PLG and PAOPA decrease haloperidol-induced increases in c-fos (RNA) and Fos (protein) [79] and vacuous chewing movements [80]. Oxotremorine-induced tremor in mice is also attenuated by PLG [3, 81]. Furthermore, PLG potentiates L-DOPA-induced responses in mice [1], and PLG and a diketopiperazine PLG peptidomimetic (**9**) Fig. (**2**) both potentiate apomorphine-induced contralateral circling behavior in 6-hydroxydopamine hemi-lesioned rats [82, 83]. In this same animal model, the highly potent ($EC_{50}$ ~ 1nM) PLG derivative, PAOPA, enhances D2-selective (i.e., apomorphine and quinpirole) and D1-selective (SKF-38393) agonist-induced responses, as well as L-DOPA-induced responses [84].

For over two decades, the therapeutic potential of PLG has been recognized with respect to its actions on dopamine receptors [2, 82, 67, 85]. However, PLG has limited bioavailability in humans and because it is a peptide it is rapidly cleared from plasma ($t_{1/2}$(elimination) = 15.2 minutes) [86]. The poor pharmacokinetics and low potency of PLG [81] has lead to the development of peptidomimetics, which may have therapeutic applications. For instance, the potentiation of agonist-induced contralateral circling behavior in 6-hydroxydopamine-lesioned rats suggests that PLG peptidomimetics might be useful antiparkinsonian adjuvants [83, 84]. In addition, the ability of PLG peptidomimetics to prevent various haloperidol-induced biochemical and behavioral changes in rodents suggests that they might be useful in the prevention of neuroleptic-induced side-effects such as tardive dyskinesia [79, 80].

## ALLOSTERIC MODULATION OF DOPAMINE RECEPTORS BY SCH202676

SCH202676 (N-(2,3-Diphenyl-1,2,4-thiadiazol-5(2H)-ylidene)methanamine (**10**), Fig. (**3**) has been described as a non-selective and noncompetitive inhibitor (i.e., it exerts neutral heterotropic cooperativity) of the binding of both agonists and antagonists to α2-adrenergic receptors [87]. It also inhibits the binding of radioligands to dopamine receptors and a variety of other biogenic amine and peptide neurotransmitter receptors [87]. In the submicromolar range ($IC_{50}$= 100 nM), SCH202676 has been shown to dose-dependently inhibit the binding of [$^3$H]SCH23390 (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) to cloned D1 receptors and [$^3$H]methylspiperone to cloned D2 dopamine receptors. While the mechanism of the binding interaction to dopamine receptors was not reported, it is presumably noncompetitive like for α2-adrenergic receptors.

**10: SCH202676**

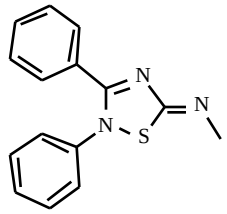



**Fig. (3).** Chemical Structure of SCH202676.

Since SCH202676 interacts with different subfamilies of GPCRs, its binding site is likely to be conserved within the heterotrimeric GPCR superfamily. However, its mode of allosteric coupling is likely to be different amongst receptor subtypes as has been shown to be the case for allosteric modulation of dopamine receptors subtypes by zinc [10, 23] and 5-amino derivatives of amiloride [47]. Since amiloride and its derivatives can both modulate GPCRs (e.g., dopamine, adrenergic and adenosine receptors) and block ion channels and ion exchangers and transporters, it would be of interest to determine whether SCH202676 also doubles as an ion channel blocker.

## HETEROTROPIC ALLOSTERIC MODULATION OF OTHER ALLOSTERIC SITES

Even though the allosteric modulators sodium, zinc and amiloride each have been shown to interact with physically

distinct receptor microdomains on the D4 dopamine receptor [18], the binding of one allosteric modulator to one allosteric site can allosterically modulate the binding of a second allosteric modulator to a second allosteric site. For example, in the presence of 120 mM sodium ions the apparent affinity ($K_i$) of zinc for its allosteric site on D4 dopamine receptors is decreased by approximately 10-fold (as measured by [$^3$H]methylspiperone/zinc inhibition curves), while MIA binding to this same receptor subtype is sodium-insensitive [18]. The effect of MIA on zinc's interactions with D4 dopamine receptors is in contrast to sodium's effect, in that MIA slightly facilitates zinc binding to D4 dopamine receptors [18]. It will be of interest to determine whether other allosteric modulators of dopamine receptors, such as PLG (and its derivatives) and SCH202676, influence the allosteric modulation of dopamine receptors by sodium, zinc and amiloride (and its derivatives) and whether they interact with some of the same receptor microdomains.

## ACKNOWLEDGEMENTS

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## ABBREVIATIONS

| | | |
|---|---|---|
| GPCR | = | G Protein-coupled receptors |
| $R_H$ | = | High affinity state |
| $R_L$ | = | Low affinity state |
| MIA | = | Methylisobutylamiloride |
| $K_D$ | = | Equilibrium dissociation constant |
| amiloride | = | 3,5-Diamino-N-(aminoiminomethyl)-6-chloro-pyrazinecarboxamide |
| PLG | = | L-proline-L-leucine-L-glycine |
| MSH | = | melanocyte stimulating hormone |
| MIF | = | MSH release-inhibiting factor |
| MRIH | = | MSH release-inhibiting hormone |
| NPA | = | propylnorapomorphine |
| ADTN | = | 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaph-thalene |
| SAR | = | structure-activity relationships |
| PAOPA | = | 3(R)-(N-L-prolylamino)-2-oxo-1-pyrrolidineac-etamide |
| $CH_2$-Phe | = | L-phenylalanine |
| SCH 2323390 | = | R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine |
| SCH 202676 | = | N-(2,3-Diphenyl-1,2,4-thiadiazol-5(2H)-ylidene) methanamine. |

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