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SYNTHESIS AND NK-2 ANTAGONIST EFFECT OF 1,6-DIPHENYL-PYRAZOLO [3,4-d]-THIAZOLO[3,2-a]4H-PYRIMIDIN-4-ONE[1]

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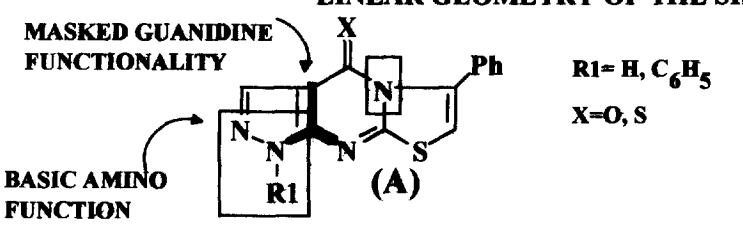
Abstract: 1,6-Diphenyl-pyrazolo[3,4-d]thiazolo[3,2-a]4H-pyrimidin-4-one caused parallel displacement of the dose-response curve to the NK-2 receptor agonist in the guinea pig trachea suggesting it acts as a competitive antagonist. It is not active in the NK-1 and NK-3 receptor binding assays. A Ca^{++} channel blocking action at the voltage-operated channels (L-type) was observed.

INTRODUCTION

Tachykinins are peptide neurotransmitters interacting with a class of G-protein-coupled receptors[2]. With few exceptions, the biological actions of tachykinins in mammals are mediated by at least three distinct cells-surface receptors, NK-1, NK-2 and NK-3, that show preferential binding for the endogenous agonists substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), respectively. There is substantial evidence to support the involvement of these neurotransmitters in nociception, neurogenic inflammation, bronchoconstriction, vasodilation, salivation and also in activation of the immune system [3] . A recent paper [4] by our laboratories identified (in a "file chemical approach") the tricyclic pyrazolothiazolopyrimidine-4-one system (**Formula A, R1=H ; X=S**) with mixed non-specific NK-1/NK-2 antagonist activity at 10 μM, as a quite promising lead to discover novel non-peptide tachykinin antagonists due either to its certain resemblance with previously reported condensed heterocycles SP antagonists e.g. linear geometry, basic amino functions and a masked guanidine functionality, or to the possibility to perform structural modifications consistent with the literature suggestions[4,5]. We describe here the NK-2 antagonist effect (apparent pA_2=7.3, equivalent to 55 nM) of 1,6-diphenyl-pyrazolo[3,4-d]thiazolo[3,2-a]4H-pyrimidin-4-one (**Formula A, R1=Ph ; X=O**) as a consequence of a slight structure modification. The change in the biological activity is consistent with the high homology of the NK receptor system[6].

LINEAR GEOMETRY OF THE SKELETON



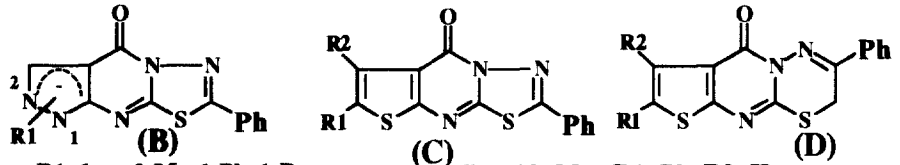
RESULTS AND DISCUSSION

There is incredible diversity amongst G-protein coupled receptors and many models have been proposed for the

interaction of the receptor with both peptide and non-peptide molecules[2,7-10]. At present our results, supported by the recent findings of other Authors[7-11], show the possibility for small non-peptide compounds with suitable functionalities to represent interesting tools to map the architecture of neurotransmitters receptors and their mechanism. Moreover previous disclosures by our laboratories showed the potential of polycondensed heterocycles, widely investigated by *F. Russo e coll.*, as antagonists of G proteins coupled receptors[12-14]. Some interesting suggestions arise from the specific action at the NK-2 receptor in comparison with the reported unsubstituted nitrogen analog (**Formula A**, R1=H; X=O,S) showing mixed NK-1/ NK-2 activity, the NK-1receptor antagonist activity being distinctly predominant[4]. Compound **8** shows Ca^{++}channel blocking action (voltage-operated (L-type) channels)[15]. The selectivity of the action at the NK-2 receptors may suggest a different calcium mobilization mechanism or two different types of calcium channel at the NK-1 and NK-2 receptors [2,11,16-18]. However, the Ca^{++}channel blocking action does not account alone for the biological activity[2,11,16,17]. Moreover, the " verapamil-like " action of the potent and selective NK-1 antagonist **CP-96,345** is not related with its NK-1 antagonist effect[17]. Another suggestion may concern a different binding of compound **8** which might not be overlapping with the binding site of the agonist (**Figure 1**). Non-peptide compounds are pharmacologically described as competitive antagonists and it is assumed, although no structural evidence is available, that they share binding sites with natural peptide ligands. However, the non-peptide compounds do not resemble the peptide agonists chemically and their mechanism of action, at a biochemical and molecular level is not clear[2,6-11]. All the data coming from the biological evaluation[19] of compound **8** (using **SR48968**[3] as a comparison) provides evidence for a selective interaction at the NK-2 system or at one effector in the stream following the agonist binding[2]. Two features seem to be important for maximum activity at the NK-2 receptors, provided that a planar rigid structure is a substantial requisite for the below listed pharmacophores: i) **AROMATIC SUBSTITUENTS.** The N1-phenyl group and the pyrazolopyrimido moiety are critical for maximum activity. The former can act as a carrier to address the NK-2 antagonist activity of compound **8** (**Figure 1**). A hydrophobic interaction of **8** or the molecule lypophilic character may play a role[3,5,20]. The isosteric oxygen-sulphur replacement , which increased the NK-1 antagonist action of the lead compound[4] (**Formula A , R1=H; X=O,S**) resulted in the inactive compound **9**, showing no significant calcium blocking action at 1 μM. A negative effect of the bulky sulphur atom on the optimal docking of the aromatic ring may play a role. Another possible explanation concerns a more favourable stabilizing interaction by the highly electronegative oxygen atom at the NK-2 receptors[7]. The removal of the phenyl ring from the position 5 to 6 of the thiazole ring gave the inactive compound **10** (10 μM). A similar effect was produced by the phenyl substituent elimination on the thiazole ring **11**; ii) **HETEROCYCLIC PORTION**. Removal of the pyrazole and the thiazole rings by the chemical different thiophene ring and the isostere thiadiazole respectively or the extension of the thiadiazole ring to the six-membered thiadiazine[21-23] **B-**

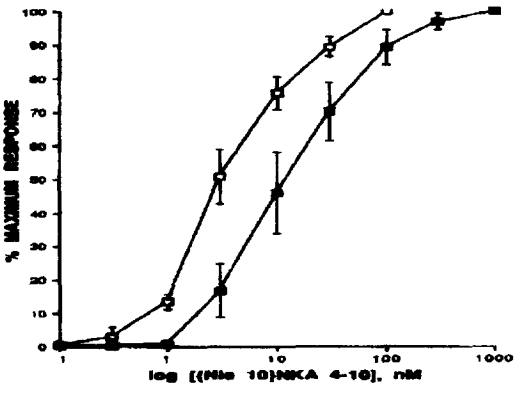
D (Figure 2) resulted in a loss or marked decrease (>>10 μM) of NK-2 activity up to a concentration of 10 μM. A similar result comes from the structural modifications **18-20**[21-25]. None of the tested compounds showed activity (>>10 μM) at the NK-1 receptor system (using **CP-96,345** [3,17,19] as a comparison).

Figure 2 Heterocyclic variations containing the thiadiazole or the thiadiazine ring



The favourable interaction of nitrogen residues in G proteins coupled receptors antagonists may agree either with a recent paper from *Verdonk et al.* about a study on interactions between aromatic $NR4^+$ groups and aromatic residues in ligand-receptor binding or with previous papers by our group. This interaction mechanism is presently known as of great relevance in molecular recognition and may be responsible for a favourable orientation of the whole antagonist molecule[12-14,26]. Future developments of the synthetic-biological work in progress supported by a computer aided approach will be subsequently reported.

Figure 1 EFFECT OF COMPOUND **8** ON NK2-RECEPTOR MEDIATED CONTRACTILE RESPONSES EVOKED BY Nle^{10}NKA (4-10) CONTRACTIONS



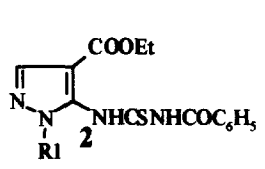
Concentration-effect curves to Nle10NKA (4-10) were conducted in the absence □ and presence ◆ of compound **8** (100 nM). The responses were inhibited by compound 8 and dose -ratios (i. e. the concentration of Nle10NKA (4-10) required to produce half maximal responses in the absence and presence of compound **8**) were calculated and used to estimate the apparent pA2 value.

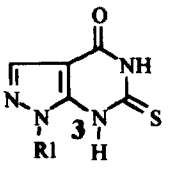
CHEMISTRY

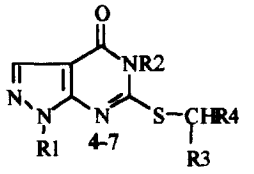
The synthesis of compounds **8,10,11** with some slight differences below listed was carried out following our previously reported procedure by a simple acid cyclodehydration in 98% sulphuric acid of the pertinent 1-phenyl-4-hydroxy-6-(phenacylthio)-pyrazolo[3,4-d]pyrimidine **5**,1-phenyl-4-hydroxy-6-[(2,2-dimethoxyethyl)thio]-pyrazolo[3,4-d]pyrimidine **6** or 1-phenyl-4-hydroxy-6-[(2-oxoethyl)thio]-pyrazolo[3,4-d]pyrimidine **7** at room perature[4] (36 hours for **8**).The latter intermediates were prepared by reaction of 1-phenyl-4-hydroxy-6-mercapto-pyrazolo[3,4-d]pyrimidine **3** (sodium or potassium salt) (1g.) with the pertinent α-bromocarbonyl compound [4], commercially available or prepared following the reported method[27], in ethanol (15-20 ml) at room tempe-

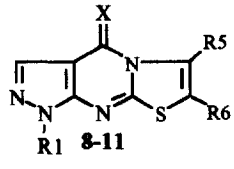
rature **5** or at reflux for 3-8 hours **6,7**. A three fold excess and some random additions of α-bromoacethaldehyde dimethylacetal during the 32 hours of reaction time were used to obtain compound **7** as a precipitate. Attempts to prepare compound **5** carrying out the reaction in acetone/Na_2CO_3 were unsuccesfull[4]. However, the bis-substituted compound **4** was converted to the tryciclic derivative **8** in the final ring closure cyclodehydration. The starting 1-phenyl-4-hydroxy-6-mercapto-pyrazolo[3,4-d]pyrimidine **3** was prepared by alkaline cyclization of 2-phenyl-N-(4-carboethoxypyrazol-3-yl) N'-benzoylthiourea **2**(1g., 0.0025 mol in 25 ml)[28]. The latter intermediate was in turn prepared by reaction of 2-phenyl-3-amino-4-carboethoxypyrazole[29] **1** (6.2g., 0.026mol) with an equimolar amount of benzoylisothiocyanate, commercially available or *in situ* prepared[30], in acetone(80 ml) at reflux for 20 hours[28]. The compound was obtained as a white solid by adding to the oil residue, a mixture ethanol-water (φ=50%) and then cooling for 48 hours. The 4-thioxo analog **11** was obtained as a precipitate from **8** (0.5 g., 0.0014 mol) by thiation reaction with Lawesson' s Reagent (3.5 g. 0.0087 mol) in anhydrous xylene(16 ml) for 66 hours[4].

Table I Physicochemical data of compounds 2-11









2-11: R1=Ph

4: R2=CH_2COPh; R3=H; R4=COPh
5: R2=R3=H; R4=COPh
6: R2=H; R3=Ph; R4=CHO
7: R2=R3=H; R4= CHO

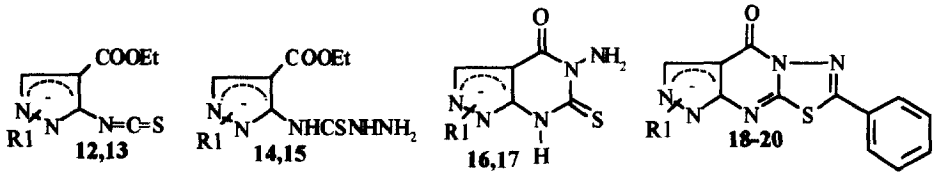
8: R5=Ph; R6=H
9: R5=H; R6=Ph
10: R5=H; R6=H
11: R5=Ph; R6=H;X=S

entry	m.p.	recr. solv.	% yield	IR(cm^{-1},KBr, neat) NH	C=O	C=S	Formula
2	125	EtOH	98	3160	1710		$C_{20}H_{18}N_4O_3S$
3	>300	EtOH	95	-	1685		$C_{11}H_8N_4OS$
4	176-8	acetic acid	45	-	1690		$C_{27}H_{20}N_4O_3S$
5	227-30	acetic acid	75	-	1690		$C_{19}H_{14}N_4O_2S$
6	94-96[31]	chrom. column	20	3380	1685		$C_{19}H_{14}N_4O_2S$
7	228-30	EtOH/H_2O	30	3240	1690		$C_{20}H_{18}N_4O_3S$ x 0.5 EtOH
8	268-70	DMF	98	-	1725		$C_{19}H_{12}N_4OS$
9	245-7	dioxane	45		1705		$C_{19}H_{12}N_4OS$
10	232-4	DMF	98		1715		$C_{13}H_8N_4OS$
11	250-2	DMF/H_2O	35	-		1241,1176	$C_{19}H_{12}N_4S_2$

The ^1H characterization of compound **6** was not possible. The compound is involved in multiple equilibria which

originates species slowly exchanging in the NMR time scale. The mass measurements are consistent with the assigned structure. Absence of any inter-NOE effect (H7-Ph1) was consistent with the linear geometry of the tricyclic derivatives **8,9-11**. The structure of the intermediate **5** was also confirmed by a chemical method, 1-phenyl-4-hydroxy-6-mercapto-pyrazolo[3,4-d]pyrimidine as control (dioxane/EtOH (φ=20%), HCl (20 ml); Δ=20 hours, then rotary evaporation)[4]. The heterocyclic variations **(Figure 2)** were prepared according to the reported methods[21-25]. In the preparation of new entries **18,19**[24] from **16,17**[21], two slight modifications concern the reaction time (1 hour) and the recovering of the compounds by pouring the reaction mixture in crushed ice. Hydrazinothioxo intermediates **14,15** were prepared by stirring compounds **12,13** with hydrazine monohydrate (98%) at room temperature for 30 min.[21]. The latter intermediates **12,13** were prepared according to the reported method prolonging the reaction time up to 5 hours [23]. Compound **20** was obtained following a previously reported method by direct condensation of 1-methyl-3-amino-4-carboethoxy pyrazole[4] with 2-chloro-5-phenyl-1,3,4-thiadiazole[25] at 180 °C in a pre-heated oil bath. The physicochemical data of newly synthesized compounds **2-20** are reported in tables **I** and **II**.

Table II Physicochemical data of the heterocyclic variations 18-20 and their intermediates 12-17



entry	R1	m.p.	recr. solv.	(%) yield	IR(cm^{-1}, KBr or nujol disc[a], neat) NH	NCS	C=O	Formula
12[31]	2-Ph	oil[a]	chrom. column	22	-	2000	1700	$C_{13}H_{11}N_3O_2S$
13[31]	2-Bz	oil[a]	chrom. column	42	-	2010	1720	$C_{14}H_{13}N_3O_2S$
14	2-Ph	163-64	EtOH	20	3190,3350	-	1720	$C_{13}H_{15}N_5O_2S$
15	2-Bz	149-50	EtOH	22	3320,3240,3160	-	1690	$C_{14}H_{17}N_5O_2S$
16	1-Ph	228-30	EtOH	25	3300,3260,3200	-	1695	$C_{11}H_9N_5OS$
17	1-Bz	203-5	EtOH/dioxane	40	3260,3130	-	1690	$C_{12}H_{11}N_5OS$
18	1-Ph	217-8	EtOH/dioxane	47			1730	$C_{18}H_{11}N_5OS$
19	1-Bz	238-40	dioxane	54			1730	$C_{19}H_{13}N_5OS$
20	2-Me	>300	DMF	40			1720	$C_{13}H_9N_5OS$

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31. **6 (Table I) :** m/z 362 ($M^{+\cdot}$, 100), 344 (20, **M** - H_2O), 334(42, M-CO), 301 (28), 274 (33), 105 (30, C_6H_5CO), 91 (74, $C_6H_5CH_2$), 77 (64, C_6H_5); Proton NMR Spectra (DMSO d-6 **8** or $CDCl_3$ **9**) **8** 8.33(s, pyrazole H3), 7.24(s, H7 thiazole),8.08(N-phenyl, 2H protons),7.59(N-phenyl, 2H protons) ,7.40 (N-phenyl 1H proton),7.3-7.5(phenyl 2H protons), 7.3-7.5(phenyl 2H protons),7.3-7.5(phenyl 1H proton); **9** 8.23(s,1H, thiazole), 8.35(s,1H, pyrazole), 7.57(d, Jvic= 9 Hz, Ar **8**), 7.46(t, 2H,Jvic= 9 Hz, Ar 8), 7.50-7.60m , (5H, Ar 1). Column chromatography of compounds **6,12,13** was performed on Silica gel 60, Merck (230-400 Mesh ASTM), system: ethyl acetate **6**, cyclohexane-ethyl acetate(φ=40%) **12,13**.

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