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Synthesis and angiotensin II receptor antagonist activity of C-linked pyrimidine derivatives

E Nicolaï[1], G Curé[1], J Goyard[1], M Kirchner[1], JM Teulon[1], A Versigny[2], M Cazes[2], A Virone-Oddos[2], F Caussade[2], A Cloarec[2]

[1]*Carpibem, 128, rue Danton, 92500 Rueil-Malmaison;*
[2]*UPSA, 128, rue Danton, 92500 Rueil-Malmaison, France*

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Summary — The synthesis and pharmacological activity of nonpeptide angiotensin II (Ang II) receptor antagonists are presented. These 3-*N*-substituted pyrimidine-4(3*H*)-one and 4-O,N,S-substituted pyrimidine derivatives represent a series of C-linked biphenyl tetrazole Ang II antagonists. *In vitro*, they displayed a high affinity for rat adrenal Ang II receptors, several compounds causing more than 60% displacement of [^{125}I]Sar^1–Ile^8–Ang II from the rat adrenal Ang II receptor at 10^{-7} M. *In vivo*, several compounds displayed a high oral antihypertensive activity in renal hypertensive rat with decreases in systolic arterial pressure (SAP) greater than 60 mmHg at 10 mg/kg. 2-[2-Methyl-4-oxo-6-*n*-propyl-5-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-3-yl]ethanol hydrochloride (compound **17**) was compared with Losartan in the renal artery-ligated rat model. It was shown that at 3 mg/kg po, **17** induced a maximal decrease in mean arterial pressure (MAP) of 60.8 mmHg, which was similar to that was observed with Losartan (maximal decrease of 60 mmHg at 3 mg/kg) with a long duration of action (greater than 16 h).

pyrimidine / antihypertensive activity / angiotensin II / angiotensin II receptor antagonist / 2'-(1*H*-tetrazol-5-yl)biphenyl

Introduction

In the late 1970s, the introduction of angiotensin-converting enzyme (ACE) inhibitors confirmed that angiotensin II (Ang II) plays an important role in regulating blood pressure and fluid and electrolyte homeostasis [1]. The recent development of Ang II receptor antagonists provided a more specific approach and stimulated a large body of research to obtain orally active antagonists. Since the discovery of Losartan (DuP 753, chart 1) [2, 3], several nonpeptide Ang II receptor antagonists have been reported [4]. The great majority of these compounds have included a biphenyltetrazole moiety, which was shown to play a critical role for the oral activity. Generally this biphenyltetrazole group was linked to a nitrogen atom of various heterocycles [4]. With the aim of discovering new Ang II receptor antagonists we developed three heterocyclic series, namely pyrazole [5], pyrimidine [6] and triazolopyrimidine [7] derivatives, in which the biphenyltetrazole is linked to a carbon atom. This work led to the discovery of a new potent orally active antagonist, UP 269-6 (chart 1), which is currently in phase-II clinical trials [8]. Recently, we have reported a structure–activity relationship study in our C-linked pyrazole series [9] (chart 1). Independently, Ciba Geigy [10, 11] and Merck [12] have patented C-linked pyrimidine derivatives as Ang II receptors antagonists. We would like to discuss here our results on the synthesis and antihypertensive activity of 3- and 4-substituted C-linked pyrimidine derivatives of formula A and B (chart 2), respectively.

Chemistry

The preparations of 4-pyrimidone **6** and 4-alkoxypyrimidine **7** derivatives were achieved as depicted in

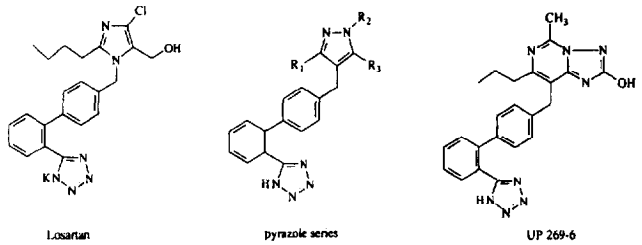


Chart 1. Structures of Losartan, pyrazole derivatives and UP 269-6.

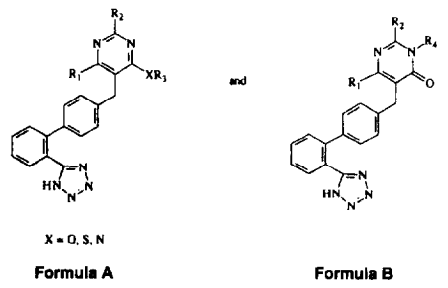
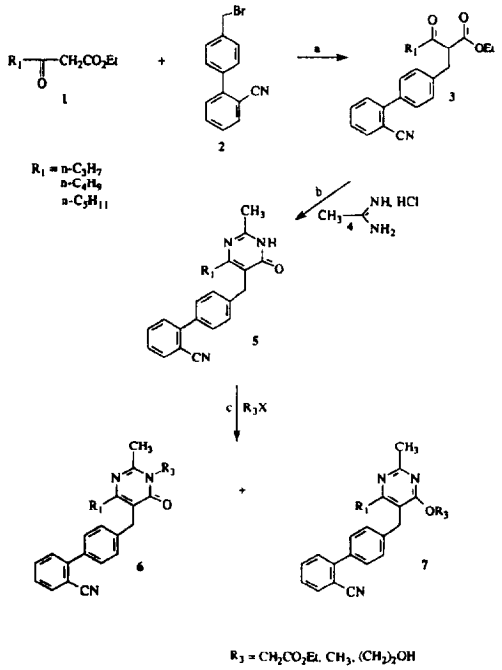


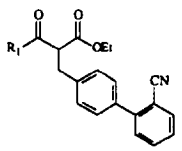
Chart 2. Structures of C-linked pyrimidine series.



Scheme 1. (a) LiCl, $(i\text{Pr})_2$NEt, THF, reflux, 15 h; (b) NaOMe, MeOH, rt 20 h, reflux 3 h; (c) NaH, DMF, 70°C, 1 h or K_2CO_3, 2-butanone, reflux 24 h.

scheme 1. The β-keto esters **1** [6] were alkylated with 4-bromomethyl-2'-cyanobiphenyl [6] **2** in THF, in the presence of lithium chloride and *N,N*-diisopropyl ethylamine according to the method of Sung-Eun and Kyu [13] to lead to the alkylated β-keto esters **3** (table I). The cyclization of **3** with amidine hydrochlorides **4** in MeOH with NaOME [14] gave the 4-pyrimidone derivatives **5** (table II). As the reaction between **3a** and formamidine hydrochloride failed to give **5e** (R_2 = H), this derivative was prepared by a three-step procedure including the synthesis of the 2-methylthio derivative **5d**. The preparations of **5d** and **5e** are depicted in scheme 2. The reaction of the

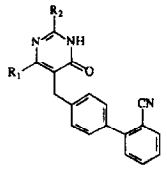
Table I. Preparation of 2-alkylated β-ketoesters **3**.



Compound	R_1	*Yield*[a] *(%)*
3a	n-C_3H_7	98
3b	n-C_4H_9	93
3c	n-C_5H_{11}	89

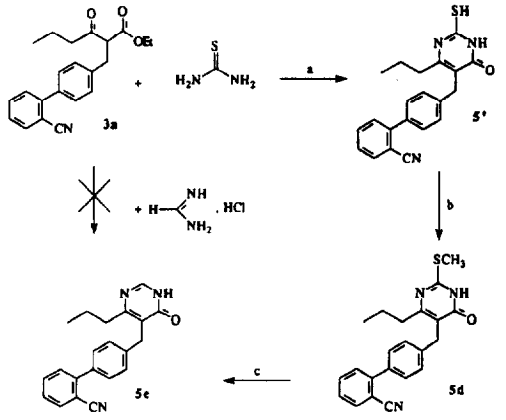
[a]Yield calculated from starting 4-bromomethyl-2'-cyanobiphenyl **2** for the crude oil used without further purification (HPLC purity 80–90%).

Table II. Preparation of pyrimidin-4(3*H*)-one **5**.



Compound	R_1	R_2	*Yield*[a] *(%)*	*Mp (°C)*
5a	n-C_3H_7	CH_3	60.8	209–210
5b	n-C_4H_9	CH_3	59	173
5c	n-C_5H_{11}	CH_3	56	162
5d	n-C_3H_7	SCH_3	36	218
5e	n-C_3H_7	H	56[b]	158

[a]Overall yield for the two steps from 4'-(bromomethyl)-2-cyanobiphenyl **2**. [b]Yield calculated from **5d**.



Scheme 2. (a) NaOMe, thiourea, MeOH, reflux 10 h; (b) ICH_3, KOH, MeOH, rt 4 h; (c) Raney Ni, diglyme, reflux 3 h.

β-keto ester **3a** with thiourea in the presence of NaOMe in MeOH at reflux led to the 2-mercaptopyrimidine derivative **5'**, which was alkylated with methyl iodide in the presence of KOH in MeOH to afford the 2-methylthiopyrimidine **5d**. Desulfurization of **5d** with Raney nickel in refluxing diglyme gave **5e**.

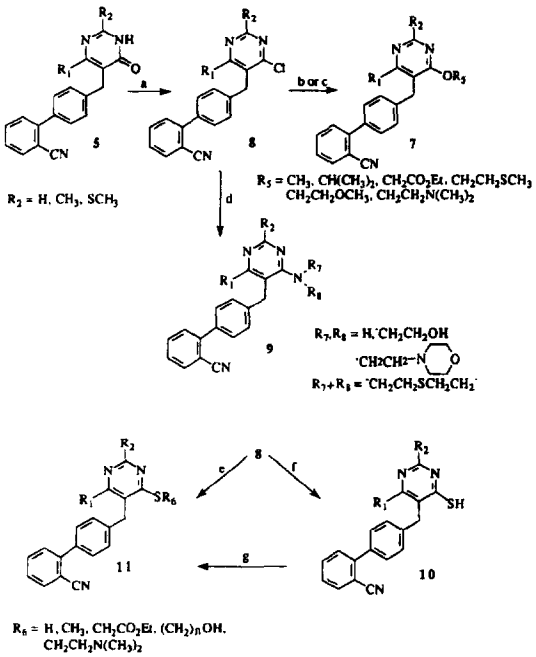
The alkylation of **5** was performed by reaction with alkyl halide (R_4X, X = Br or Cl) in the presence of NaH in DMF or K_2CO_3 in 2-butanone and led to a mixture of *N*-alkylated derivatives **6** and *O*-alkylated derivatives **7** which were easily separated by chromatography on silica gel. The predominance of *N*-alkylated derivatives **6** upon *O*-alkylated derivatives **7** depended on the experimental conditions and reagents. When ethyl bromoacetate or an alkyl halide with NaH/DMF were used, *N*-alkyl derivatives were obtained predominantly. In contrast when 2-bromoethyl acetate was used in the same conditions, *O*-alkyl derivatives **7** prevailed. When 2-bromoethanol was used with K_2CO_3 and 2-butanone, the *N*-alkyl and *O*-alkyl derivatives were obtained in the ratio 3:2.

Some 4-alkoxy, 4-amino, 4-mercapto and 4-alkylthiopyrimidine derivatives, **7**, **9**, **10** and **11**, respectively, were prepared according to scheme 3. Treatment of **5** with $POCl_3$ at reflux [15] led to the 4-chloropyrimidine derivatives **8** (table III) which reacted with alcohols R_5OH in THF with NaH, MeOH or *i*-propanol in the presence of one equivalent of Na (when R_5 = CH_3 or *i*-C_3H_7) to lead to 4-oxy-substituted pyrimidines **7**. Reaction of **8** with alkylamines HNR_7R_8 in refluxing xylene afforded 4-alkylamino derivatives **9**. The preparation of 4-thio derivatives **11** was achieved in two different ways. The first consisted of the direct reaction of 4-chloropyrimidines **8** with thiols (HRS_6) in EtOH in the presence of a base such as NaOAc or in 2-butanone with K_2CO_3. In the second method, we used a two-step procedure including the intermediate formation of the 4-mercaptopyrimidines **10**, which were obtained by heating **8** with thiourea in refluxing acetone with a catalytic amount of HCl. Alkylation of **10** with alkyl halide in EtOH in the presence of NaOH or Na, led to the 4-alkylthio pyrimidines **11**.

The target tetrazole derivatives were synthesized as depicted in scheme 4 by treatment of the corresponding cyano derivatives with Me_3SnN_3 in refluxing xylene or toluene and subsequent cleavage of the intermediary *N*-trimethylstannyl tetrazole derivatives with gaseous HCl in THF.

Results and discussion

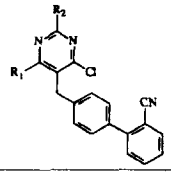
The *in vitro* affinity of the compounds was measured by their ability to inhibit the specific binding of [^{125}I] Sar^1–Il^8–Ang II from rat adrenal Ang II receptors at 10^{-5} M and 10^{-7} M. The compounds (tables IV–VII)



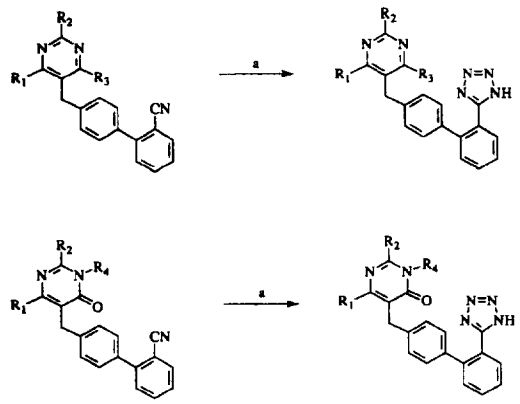
Scheme 3. (a) $POCl_3$, 100°C, 6 h; (b) R_5OH, NaH, THF, reflux 7 h; (c) R_5OH, Na, rt 3 h; (d) R_7R_8NH (2.5 eq), xylene, reflux, 16 h; (e) R_6SH, K_2CO_3, 2-butanone, reflux 8 h; (f) thiourea, acetone, conc HCl (cat), reflux 6 h; (g) R_6X, K_2CO_3, acetone, reflux 3 h.

were tested orally in renal artery-ligated hypertensive rats [16], a high renin model, at 10, 3 or 1 mg/kg by either the tail-cuff method [17] or by a direct method [18]. Results are expressed as change in arterial blood pressure (systolic arterial pressure (SAP) for the tail-cuff method and mean arterial pressure (MAP) for the direct method).

Table III. 4-Chloropyrimidine derivatives **8**.



Compound	R_1	R_2	*Yield (%)*	*Mp (°C)*
8a	*n*-C_3H_7	CH_3	98	102
8b	*n*-C_4H_9	CH_3	95	75
8c	*n*-C_5H_{11}	CH_3	92	oil
8d	*n*-C_3H_7	SCH_3	84	88
8e	*n*-C_3H_7	H	91	95



Scheme 4. (a) Me_3SnN_3, toluene, reflux 30 h.

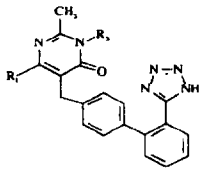
The major purpose of our work was to identify orally active compounds. Therefore the *in vitro* data only concern the percentage displacement at 10^{-5} M and 10^{-7} M and do not allow us to rationally discuss the *in vitro* SAR.

The results in table IV allow us to discuss the influence of the substituents at the 3- and 6-positions of the *N*-substituted pyrimidines derivatives. *In vitro*, all compounds displayed a high affinity causing more than 60% displacement of the radioligand [^{125}I] Sar^1–Ile^8–Ang II from the rat adrenal receptor. The presence of a substituent at the 3-position (R_3 = H or CH_3, CH_2CO_2Et, CH_2CH_2OH) did not result in any significant variation of the affinity.

In vivo, the oral activities of **12** and **13** (R_3 = CH_2CO_2Et), **14** (R_3 = CH_3) and **17** (R_3 = CH_2CH_2OH) showed that a substitution at the 3-position led to equipotent derivatives to the unsubstituted **18** and **19** (R_3 = H). Thus, the nature of the 3-substituent in the 4-pyrimidone series does not seem to have a marked influence on the oral antihypertensive activity at 10 mg/kg. On the contrary the nature of R_1 (6-position of the pyrimidone) was critical for the oral activity. Comparison among **18** (R_1 = *n*-C_3H_7), **19** (R_1 = *n*-C_4H_9) and **20** (R_1 = *n*-C_5H_{11}) showed that the oral activity was optimal when the alkyl side chain was an *n*-propyl or an *n*-butyl while it decreased markedly when this chain was an *n*-pentyl. This was confirmed by comparison between **14** and **15**.

Table V presents the activities of 4-alkoxy derivatives. In the series, comparison between **22** and **24** indicated that the presence of a methyl group at the 2-position of the pyrimidine ring is favorable for *in vitro*

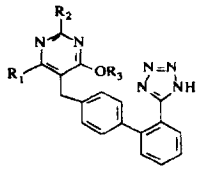
Table IV. 3-Substituted pyrimidin-4(3*H*)-one derivatives.



Compound	R_1	R_3	*Method*	*Yield*[a] *(%)*	*Mp (°C)*	*Formula*[b]	*In vitro activity % displacement*[c]		*Oral activity change in BP (mmHg)*[d]
							10^{-5} M	*10^{-7} M*	*(10 mg/kg)*
12	*n*-C_3H_7	CH_2CO_2Et	A	32	164–165	$C_{26}H_{28}N_6O_3$	94 ± 1	67 ± 2	–54.4 ± 8.1
13	*n*-C_4H_9	CH_2CO_2Et	A	37	170–171	$C_{27}H_{30}N_6O_3$	80 ± 1	60 ± 1	–105.6 ± 9.6
14	*n*-C_4H_9	CH_3	B	43	192–194	$C_{24}H_{26}N_6O$	68 ± 3	62 ± 2	–72.8 ± 14.6
15	*n*-C_5H_{11}	CH_3	B	48	215–217[e]	$C_{25}H_{28}N_6O$	68 ± 2	59 ± 1	–49.6 ± 9.1
16	*n*-C_5H_{11}	$(CH_2)_2OH$	C	8	152–153	$C_{26}H_{30}N_6O_2$	70 ± 1	60 ± 1	NT[f]
17	*n*-C_3H_7	$(CH_2)_2OH$	D	12	240–241[e]	$C_{24}H_{26}N_6O_2$	72 ± 2	62 ± 3	–95.5 ± 18.4
18	*n*-C_3H_7	H	F	52	204–206	$C_{22}H_{22}N_6O$	65 ± 1	60 ± 1	–69.6 ± 5.6
19	*n*-C_4H_9	H	F	54	248–249	$C_{23}H_{24}N_6O$	71 ± 2	67 ± 1	–70.8 ± 12.9
20	*n*-C_5H_{11}	H	F	48	195–196	$C_{24}H_{26}N_6O$	68 ± 3	60 ± 1	–39.2 ± 5.8

[a]Overall yield for all steps described in the method. [b]All elemental analyses for C, H, and N were within ± 0.4% of the calculated values unless otherwise noted. [c]Percent displacement of [^{125}I]Sar^1–Ile^8–Ang II bound to rat adrenal membranes by the compounds at 10^{-5} and 10^{-7} M; values are mean ± SEM of three determinations. [d]SAP values were determined by the tail-cuff method in renal artery-ligated rat and represent the mean ± SEM of 3–7 determinations. [e]Hydrochloride. [f]Not tested.

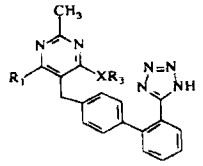
Table V. 4-O-Substituted pyrimidine derivatives.



Compound	R_1	R_2	R_3	Method	Yield[a] (%)	Mp (°C)	Formula[b]	In vitro activity % displacement[c] 10^{-5} M	In vitro activity % displacement[c] 10^{-7} M	Oral activity change in BP (mm Hg)[d] (10 mg/kg)
21	n-C_3H_7	CH_3	$(CH_2)_2OH$	D	8	154–155	$C_{24}H_{26}N_6O_2$	73 ± 1	64 ± 2	–59.7 ± 8.1
22	n-C_3H_7	CH_3	CH_3	F	38	132–134	$C_{23}H_{24}N_6O$	71 ± 3	51 ± 1	–82.8 ± 15.0[e]
23	n-C_4H_9	CH_3	CH_3	F	42	148–149	$C_{24}H_{26}N_6O$	69 ± 2	53 ± 2	–99.5 ± 2.2
24	n-C_3H_7	H	CH_3	F	35	166–168	$C_{22}H_{22}N_6O$	70 ± 1	35 ± 1	–67.2 ± 6.4
25	n-C_3H_7	CH_3	i-C_3H_7	F	43	190–191	$C_{25}H_{28}N_6O$	70 ± 3	38 ± 1	–23.7 ± 6.9
26	n-C_3H_7	CH_3	CH_2CO_2Et	G	41	149–150	$C_{26}H_{28}N_6O_3$	73 ± 2	62 ± 1	–104.8 ± 14.6
27	n-C_4H_9	CH_3	CH_2CO_2Et	G	46	133–135	$C_{27}H_{30}N_6O_3$	62 ± 2	37 ± 1	NT[f]
28	n-C_3H_7	CH_3	$(CH_2)_2OCH_3$	G	43	169–170	$C_{25}H_{28}N_6O_2$	74 ± 1	60 ± 2	–33.5 ± 16.1
29	n-C_4H_9	CH_3	$(CH_2)_2OCH_3$	G	39	128–129	$C_{25}H_{28}N_6O_2$	73 ± 2	53 ± 1	–39.0 ± 6.7
30	n-C_3H_7	CH_3	$(CH_2)_2SCH_3$	G	45	121–122	$C_{25}H_{28}N_6OS$	69 ± 1	46 ± 1	–20.5 ± 5.7
31	n-C_4H_9	CH_3	$(CH_2)_2SCH_3$	G	44	122–123	$C_{26}H_{30}N_6OS$	67 ± 2	38 ± 1	NA[g]
32	n-C_3H_7	CH_3	$(CH_2)_2N(CH_3)_2$	G	47	175–176	$C_{26}H_{31}N_7O$	68 ± 1	33 ± 1	–32.1 ± 11.1
33	n-C_4H_9	CH_3	$(CH_2)_2N(CH_3)_2$	G	43	166–167	$C_{27}H_{33}N_7O$	66 ± 2	24 ± 2	–24.2 ± 10.1

[a–d]See footnotes of table IV. [e]Tested at 30 mg/kg. [f]Not tested. [g]Not active.

Table VI. 4-*S*-substituted and 4-*N*-substituted pyrimidine derivatives.



Compound	R_1	XR_3	Method	Yield[a] (%)	Mp (°C)	Formula[b]	In vitro activity % displacement[c] 10^{-5} M	In vitro activity % displacement[c] 10^{-7} M	Oral activity change in BP (mmHg)[d] (10 mg/kg)
34	n-C_3H_7	$S(CH_2)_2OH$	H	28	186–189	$C_{24}H_{26}N_6OS$	70 ± 1	62 ± 2	–54.9 ± 5.5
35	n-C_4H_9	$S(CH_2)_2OH$	H	31	161–162	$C_{25}H_{28}N_6OS$	63 ± 1	40 ± 1	NT[e]
36	n-C_3H_7	$S(CH_2)_3OH$	J	43	157–158	$C_{25}H_{28}N_6OS$	66 ± 1	58 ± 2	–36.5 ± 8.9
37	n-C_4H_9	$S(CH_2)_2N(CH_3)_2$	H	32	187–188	$C_{27}H_{33}N_7S$	66 ± 2	52 ± 1	–25.4 ± 11.5
38	n-C_3H_7	SCH_2CO_2Et	H	35	137–138	$C_{26}H_{28}N_6O_2S$	69 ± 3	62 ± 1	–91.7 ± 24.3[f]
39	n-C_3H_7	SH	I	25	247–248	$C_{22}H_{22}N_6S$	68 ± 1	63 ± 1	–87.8 ± 22.8
40	n-C_3H_7	SCH_3	J	50	173–174	$C_{23}H_{24}N_6S$	79 ± 3	56 ± 1	–56.1 ± 15.3
41	n-C_3H_7	$NH(CH_2)_2OH$	K	26	149–150	$C_{24}H_{27}N_7O$	68 ± 1	54 ± 1	NT
42	n-C_3H_7	$N(CH_2)_2$	K	31	270–272	$C_{28}H_{34}N_8O$	69 ± 1	55 ± 1	NT
43	n-C_3H_7	–	K	35	240–241[g]	$C_{26}H_{29}N_7S$	71 ± 2	39 ± 1	

[a–d]See footnotes of table IV. [e]Not tested. [f]Tested at 30 mg/kg. [g]Hydrochloride.

Table VII. Oral antihypertensive activity at 3 and 1 mg/kg.

Compound	*Oral antihypertensive activity change in BP (mmHg)*[a]	
	1 mg/kg	*3 mg/kg*
15	NA[b]	–30.4 ± 11.8
17	–22.5 ± 4.7	–60.8 ± 4.2
18	–53.6 ± 18.8	NT[c]
19	–22.8 ± 4.4	–53.9 ± 9.0
21	NA	NT[c]

[a]MAP values were determined by the direct method [15] in renal artery-ligated rats and represent the mean ± SEM of 3–7 determinations. [b]NA: not active. [c]NT: not tested.

activity but has no dramatic effect on the oral activity. This is in contrast to what was observed in our triazolopyrimidine series in which the presence of a methyl group at this position was shown to be critical for oral activity [8]. On the other hand, the nature of the substitution at the oxygen atom seems to be critical. The substitutions with a methyl group (**22** and **23**) or an ethylacetate (**26**) led to potent derivatives whereas substitution with a hydroxyethyl (**21**) decreased slightly the oral activity. Substitution with alkoxyalkyl (**28** and **29**), alkylthioalkyl (**30** and **31**) or a 2-(dimethyl amino)ethyl group (**32** and **33**) led to a dramatic drop in both the *in vitro* and *in vivo* activities. Comparison between **22** (R_3 = CH_3) and **25** (R_3 = i-C_3H_7) showed that a short alkyl chain is better than a bulkier branched one, since the O-i-C_3H_7 derivative was poorly active at 10 mg/kg.

The 4-alkylthio derivatives presented in table VI generally displayed high affinities. *In vivo*, the best compound was the unsubstituted 4-mercapto derivative **39** (XR_3 = SH) which was equipotent to the best 3-*N*- and 4-*O*-substituted derivatives. A substitution at the 4-*S* atom was rather unfavorable for the oral activity especially with bulky groups such as 3-hydroxypropyl (**36**) or 2-(dimethylamino)ethyl (**37**). Substitution with an ethylacetate (**38**) was less favorable than in the case of 4-*O*-substituted derivatives (see **26** in table V).

In conclusion, the best substitutions at the 6-position were n-C_3H_7 and n-C_4H_9 and a longer alkyl chain was not favorable. As regards the nature of the substitution at 3- or 4-positions, H, CH_3, CH_2CH_2OH and CH_2CO_2Et appear to be optimal for oral activity, the CH_2CO_2Et group being beared preferentially by a 3-*N* or 4-*O* atom rather than by a 4-*S* atom and the CH_2CH_2OH group being preferentially linked to the 3-*N* atom.

Some compounds were tested for antihypertensive activity at lower doses (3 or 1 mg/kg). The results (table VII) showed that the unsubstituted pyrimidine **18** displayed a very high oral activity at 1 mg/kg with a decrease in MAP of 53.6 ± 18.8 mmHg. Comparison between **15**, **18** and **19** showed that the n-propyl chain was optimal for oral activity and that a longer alkyl side chain led to a loss of oral activity (n-C_3H_7 > n-C_4H_9 > n-C_5H_{11}). Comparison between **17** and **21** confirmed the superiority of the 3-hydroxyethyl (**17**) over the 4-(hydroxyethoxy)pyrimidine derivative (**21**) when tested at 1 mg/kg. As an illustration the oral antihypertensive activity of **17** was compared to that of Losartan at 3 mg/kg in renal artery-ligated rat model.

Oral antihypertensive activity of UP 243-38

In studies designed to determine the duration of action of UP 243-38 and compare its antihypertensive activity with Losartan (DuP 753), the two compounds were given orally at the dose of 3 mg/kg in conscious renal artery-ligated rats. Continuous measurements of blood pressure by the direct method [18] were performed for at least 16 h after drug administration. Administration of 3 mg/kg of either UP 243-38 or Losartan to these renin-dependent hypertensive rats resulted in a significant antihypertensive response (fig 1).

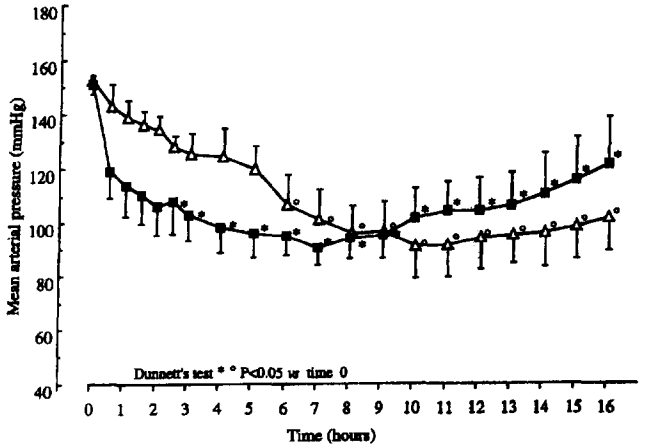


Fig 1. Oral antihypertensive activity of UP 243-38 (derivative **17**) (■) and Losartan (Δ). Time-course of the antihypertensive activity of UP 243-38 (derivative **17**), Losartan (DuP 753) in the conscious renal hypertensive rats. Hypertension in the rat was developed by renal artery ligation as described [16]. Compounds were administered orally by gavage at 3 mg/kg, 7 d after renal artery ligation. The vehicle was an aqueous suspension containing gum arabic, Tween 80 and NaCl. Values represent the mean ± SEM (n = 5–6 rats/group). An asterisk indicates a difference from pretreatment values (P < 0.05).

The maximal decrease in arterial blood pressure was 60.8 and 60.0 mmHg for UP 243-38 and Losartan, respectively. The onset of the antihypertensive response of Losartan was slower than that of UP 243-38 with a maximum effect occurring at 7 h for UP 243-38 and at 10 h for Losartan. The duration of antihypertensive action of the two compounds was similar to the response lasting for at least 16 h. In conclusion we can assume that UP 243-38 is equipotent to Losartan with a slightly different pharmacokinetic pattern.

Experimental protocols

Chemistry

^{1}H-NMR spectra were measured at 200 MHz on a Bruker 200 spectrometer and recorded in $CDCl_3$ or DMSO-d_6. Chemical shifts were reported in δ (ppm) units relative to internal reference Me_4Si. Melting points were recorded on an Electrothermal digital capillary melting point apparatus and are uncorrected. Chromatography was performed on silica gel (mesh 70–230) using indicated solvent mixture. Elemental analyses were obtained by using a Carlo Erba MOD-106 elemental analyser. HPLC experiments were performed on a Varian liquid chromatograph with UV detector and suitable integration system (inverse phase C18 column).

Starting materials were commercially available or their preparation could be found in reference [6].

Ethyl 2-[(2'-cyanobiphenyl-4-yl)methyl]-3-oxohexanoate **3a**

N,N-Diisopropylethylamine (1117 ml, 6.4 mol) and LiCl (134.6 g, 3.17 mol) were added to a solution of 4'-(bromomethyl)-2-cyanobiphenyl [6] (**2**, 863.7 g, 3.17 mol) and ethyl butyrylacetate (752 ml, 4.76 mol) in 3900 ml THF. The mixture was refluxed for 15 h and then concentrated *in vacuo*. The residue was taken up with water and extracted with AcOEt. The organic layer was washed carefully with 1 N HCl solution and then water, dried over $MgSO_4$ and evaporated *in vacuo*. The oily brownish residue was heated at 130°C under 20 mmHg in order to remove the residual starting materials to yield **3a** (1083 g, 98%) as a crude brown oil used without further purification for the next step (HPLC purity: 85.5%; 4.3% of dialkylated derivative was detected); ^{1}H-NMR ($CDCl_3$) δ: 7.75 (d, 1H, J = 8 Hz), 7.63 (t, 1H, J = 8 Hz), 7.49–7.39 (m, 4H), 7.30 (d, 2H, J = 8 Hz), 4.16 (q, 2H, J = 7 Hz), 3.84 (t, 1H, J = 7.5 Hz), 3.22 (d, 2H, J = 7.5 Hz), 2.65–2.29 (m, 2H), 1.57 (sext, 2H, J = 7.4 Hz), 1.22 (t, 3H, J = 7.5 Hz), 0.86 (t, 3H, J = 7.4 Hz).

All compounds of formula **3** were prepared according to this procedure and are listed in table I.

5-[(2'-Cyanobiphenyl-4-yl)methyl]-2-methyl-6-n-propylpyrimidin-4(3H)-one **5a**

An NaOMe solution, prepared from sodium (120.6 g, 5.24 mol) in 1.5 l MeOH, was added dropwise to a solution of acetamidine hydrochloride (466 g, 4.92 mol) in 4.3 l MeOH. The mixture was stirred for 15 min at room temperature and **3a** (1148 g, 3.28 mol; HPLC purity 85.5%) in solution in 1.15 l MeOH was added rapidly. After stirring for 20 h at room temperature the reaction mixture was refluxed for 3 h and concentrated *in vacuo* (5 l MeOH were distilled off). Water (4 l) and diisopropyl ether (1 l) were added to the mixture and after vigorous stirring the crystals were filtered off and washed with water and diisopropyl ether, dried and recrystallized in 3.5 volumes of 2-methoxyethanol to give **5a** (684.5 g, 60.8%), mp 209–210°C; ^{1}H-NMR ($CDCl_3$) δ: 7.74 (d, 1H, J = 7.6 Hz), 7.58 (t, 1H, J = 7.6 Hz), 7.44–7.35 (m, 6H), 3.97 (s, 2H), 2.60 (t, 2H, J = 7.5 Hz), 2.40 (s, 3H), 1.62 (sext, 2H, J = 7.5 Hz), 0.94 (t, 3H, J = 7.5 Hz).

Compounds **5a–c** were synthesized by this method starting from appropriate 2-alkylated β-ketoesters **3** and acetamidine hydrochloride **4**. Corresponding data are shown in table II.

5-[(2'-Cyanobiphenyl-4-yl)methyl]-2-methylthio-6-n-propylpyrimidin-4(3H)-one **5d**

Thiourea (18.9 g, 248 mmol) was added to a solution of sodium (5.7 g, 248 mmol) in 150 ml MeOH. The mixture was stirred for 5 min and **3a** (58 g, 166 mmol) was added. After refluxing for 10 h, the mixture was cooled and the MeOH was evaporated off *in vacuo*. The residue was taken up with water and washed with Et_2O, the aqueous layer was neutralized by adding dilute HCl and the obtained crystals were filtered off, washed with water and Et_2O to give 26 g of 2-mercapto derivative (mp 191°C) which were dissolved in a solution of KOH (5 g, 89 mmol) in 100 ml MeOH. Iodomethane (6 ml, 96 mmol) was added and the mixture was stirred for 4 h at room temperature. The crystals were collected by filtration, washed with water and Et_2O to give **5d** (23 g, 36% from **2**), mp 218°C; ^{1}H-NMR (DMSO-d_6) δ: 7.76–7.60 (m, 2H), 7.50–7.39 (m, 4H), 7.31 (d, 2H, J = 8 Hz), 3.88 (s, 2H), 2.56–2.47 (m, 5H), 1.64 (sext, 2H, J = 7.5 Hz), 0.89 (t, 3H, J = 7.5 Hz).

5-(2'-Cyanobiphenyl-4-yl)methyl-6-n-propylpyrimidin-4(3H)-one **5e**

Raney nickel (60 g) was added to a solution of **5d** (29 g, 77 mmol) in 250 ml diglyme. The mixture was heated to reflux for 3 h and after cooling, the catalyst was filtered off and washed with EtOH. The filtrate was evaporated *in vacuo* and the residue was chromatographed on silica gel with a mixture of acetone/$CHCl_3$ (2:8) to give **5e** (14.2 g, 56%), mp 158°C; ^{1}H-NMR (DMSO-d_6) δ: 8.17 (s, 1H), 7.92 (d, 1H, J = 8 Hz), 7.77 (t, 1H, J = 8 Hz), 7.58 (t, 2H, J = 8 Hz), 7.48 (d, 2H, J = 8 Hz), 7.33 (d, 2H, J = 8 Hz), 3.88 (s, 2H), 2.53 (t, 2H, J = 7.5 Hz), 1.64 (sext, 2H, J = 7.5 Hz), 0.87 (t, 3H, J = 7.5 Hz).

4-Chloro-5-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-6-n-propyl pyrimidine **8a**

Compound **5a** (104.8 g, 305 mmol) was added portionwise to 167 ml $POCl_3$ in 30 min; a slightly exothermic reaction occurred and temperature rose to 43°C. On completion of the addition, the reaction mixture was allowed to return to room temperature and was slowly heated to 100°C. After 6 h at this temperature the excess of $POCl_3$ was evaporated *in vacuo* and 100 ml toluene was added. The solvent was removed *in vacuo* and the residue taken up in 500 ml CH_2Cl_2 and washed with water. The organic layer was then dried over $MgSO_4$ and evaporated to give **8a** (108.1 g, 98%), mp 102°C; ^{1}H-NMR ($CDCl_3$): δ 7.75 (d, 1H, J = 7.7 Hz), 7.64 (t, 1H, J = 7.7 Hz), 7.51–7.43 (m, 4H), 7.21 (d, 2H, J = 8 Hz), 4.23 (s, 2H), 2.77–2.69 (m, 5H), 1.65 (sext, 2H, J = 7.5 Hz), 0.94 (t, 3H, J = 7.5 Hz).

All compounds **8a–e** were prepared according to this procedure and corresponding data are shown in table III.

Method A

Ethyl [5[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-4-oxo-6-n-propylpyrimidin-3-yl]acetate **6a**. NaH (60% in oil, 0.6 g,

15 mmol) was added portionwise to a solution of **5a** (5 g, 14.6 mmol) in 50 ml DMF. The mixture was stirred for 30 min at 50°C, cooled to room temperature and ethyl bromoacetate (2 ml, 18 mmol) was added. After stirring for 3 h at room temperature and for 1 h at 70°C, the solvent was evaporated off *in vacuo*. The residue was taken up with water and extracted with Et_2O. The ethereal layer was evaporated to dryness *in vacuo* and the oily residue crystallized in a mixture of diisopropyl ether/acetone (95:5) give **6a** (3.5 g, 56%), mp 98°C.

Ethyl [2-methyl-4-oxo-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl]methyl]pyrimidin-3-yl]acetate ***12***. Trimethyltin azide (2 g, 10.1 mol) was added to a solution of **6a** (3.5 g, 8.2 mmol) in 30 ml toluene. The mixture was refluxed for 30 h. After cooling the mixture was extracted with an NaOH solution. The aqueous layer was acidified by bubbling of SO_2 and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, concentrated *in vacuo* and the residue was chromatographed on silica gel with $CHCl_3$/MeOH (8:2) to give **12** (2 g, 57%), mp 164–165°C; ^{1}H-NMR ($CDCl_3$): δ 7.88 (d, 1H, J = 7.3 Hz), 7.53–7.36 (m, 3H), 7.04–6.92 (m, 4H), 4.68 (s, 2H), 4.18 (q, 2H, J = 7 Hz), 3.78 (s, 2H), 2.44 (t, 2H, J = 7.5 Hz), 2.36 (s, 3H), 1.56 (sext, 2H, J = 7.5 Hz), 1.23 (t, 3H, J = 7 Hz), 0.87 (t, 3H, J = 7.5 Hz).

Method B

6-n-Butyl-5-[2'-(cyanobiphenyl-4-yl)methyl]-2,3-dimethyl-pyrimidin-4(3H)-one ***6b***. NaH (60% in oil, 0.8 g, 20 mmol) was added portionwise to a solution of **5b** (6.5 g, 18 mmol) in 100 ml DMF. The mixture was heated to 50°C for 1 h and iodomethane (2 ml, 30 mmol) was added. The mixture was stirred for 6 h at 50°C, cooled and the solvent was removed *in vacuo*. The residue was taken up with water and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The oily residue was chromatographed on silica gel with AcOEt/cyclohexane (9:1) to give **6b** (5.5 g, 82%) as an oil; ^{1}H-NMR ($CDCl_3$): δ 7.74 (d, 1H, J = 7.3 Hz), 7.61 (t, 1H, J = 7.3 Hz), 7.50–7.32 (m, 6H), 3.96 (s, 2H), 3.96 (s, 2H), 3.53 (s, 3H), 2.59 (t, 2H, J = 7.5 Hz), 2.52 (s, 3H), 1.63–1.25 (m, 4H), 0.90 (t, 3H, J = 7.5 Hz).

6-n-Butyl-2,3-dimethyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl]pyrimidin-4(3H)-one ***14***. Trimethyltin azide (2.5 g, 12.6 mmol) was added to a solution of **6b** (3.4 g, 9.1 mmol) in 100 ml xylene. The mixture was heated to reflux for 30 h. The solid material was collected and taken up in 100 ml THF. Gaseous HCl was bubbled into the resulting suspension for 1 h and the mixture was allowed to stand to room temperature overnight. The solvent was removed *in vacuo* and the residue was dissolved in a dilute NaOH solution and washed with Et_2O. Then SO_2 was bubbled through the aqueous layer until pH 5.5 and the crystals obtained were collected and chromatographed on silica gel with CH_2Cl_2/acetone (9:1) to give **14** (2.1 g, 52%), mp 192–193°C; ^{1}H-NMR (DMSO-d_6): δ 7.70–7.48 (m, 4H), 7.11 (d, 2H, J = 7.9 Hz), 3.79 (s, 2H), 3.43 (s, 3H), 2.48–2.40 (m, 5H), 1.50–1.16 (m, 4H), 0.82 (t, 3H, J = 7.5 Hz).

Method C

2-[[6-n-Pentyl-5-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-pyrimidin-4-yl]oxy]ethyl acetate ***7c*** *and 2-[6-n-pentyl-5-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-4-oxopyrimidin-3-yl]-ethyl acetate* ***6c***. NaH (60% in oil, 1 g, 25 mmol) was added to a solution of **5c** (8 g, 21 mmol) in 100 ml DMF. The mixture was heated to 50°C under stirring for 1 h and 2-bromoethyl acetate (4.5 g, 25 mmol) was added. The mixture was heated for an additional 6 h to 50°C and the solvent was evaporated off *in vacuo*. The residue was taken up in AcOEt and water. The organic layer was separated and concentrated *in vacuo* to give an oily residue which was chromatographed on silica gel with cyclohexane/AcOEt (5:5) to afford **7c** (4.6 g, 48%) oil; ^{1}H-NMR ($CDCl_3$): δ 7.71 (d, 1H, J = 7.3 Hz), 7.59 (t, 1H, J = 7.3 Hz), 7.45–7.38 (m, 4H), 7.21 (d, 2H, J = 8 Hz), 4.58–4.52 (m, 2H), 4.48–4.42 (m, 2H), 3.98 (s, 2H), 2.68 (t, 2H, J = 8Hz), 2.54 (s, 3H), 1.98 (s, 3H), 1.63–1.45 (m, 2H), 1.31–1.21 (m, 4H), 0.80 (t, 3H, J = 8 Hz).

Further elution with cyclohexane/AcOEt (2:7) allowed us to obtain **6c** (3.3 g, 34.5%), oil; ^{1}H-NMR ($CDCl_3$): δ 7.68 (d, 1H, J = 7.3 Hz), 7.54 (t, 1H, J = 7.3 Hz), 7.42–7.28 (m, 6H), 4.39–4.31 (m, 2H), 4.28–4.19 (m, 2H), 3.89 (s, 2H), 2.58–2.48 (m, SH), 2.00 (s, 3H), 1.64–1.42 (m, 2H), 1.32–1.16 (m, 4H), 0.80 (t, 3H, J = 8 Hz).

3-[6-n-Pentyl-2-methyl-4-oxo-5-[[2'(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-3-yl]ethanol ***16***. Trimethyltin azide (1.6 g, 8.1 mmol) was added to a solution of **6c** (3.3 g, 7 mmol) in 100 ml toluene. The mixture was refluxed for 30 h. After cooling, the solvent was evaporated off *in vacuo* and the residue was chromatographed on silica gel with a mixture AcOEt/cyclohexane (5:5) to give 1.1 g of an oily compound. The latter was dissolved in 20 ml MeOH and 10 ml water and NaOH (0.3 g, 7.5 mmol) was added. The mixture was stirred for 24 h at room temperature, the solvent was evaporated and 50 ml water was added. The aqueous layer was washed with AcOEt acidified with SO_2 (pH 5.5) and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to give a residue which crystallized in acetone to afford **16** (0.8 g, 24%), mp 152–153°C; ^{1}H-NMR (DMSO-d_6): δ 7.71–7.49 (m, 4H), 7.11 (d, 2H, J = 7.9 Hz), 6.97 (d, 2H, J = 7.9 Hz), 5.04 (br s, 1H), 4.03 (m, 2H), 3.79 (s, 2H), 3.63 (m, 2H), 2.54 (s, 3H), 2.42 (t, 2H, J = 7.5 Hz), 1.42 (m, 2H), 1.21 (m, 4H), 0.80 (t, 3H, J = 7.5 Hz).

Method D

2-[5-[2'-(Cyanobiphenyl)methyl]-2-methyl-4-oxo-6-n-propylpyrimidin-3-yl]ethanol ***6d*** *and 2-[[5-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-6-n-propylpyrimidin-4-yl]oxy]ethanol* ***7d***. K_2CO_3 (4 g, 28.9 mmol) and 2-bromoethanol (8 ml, 42.3 mmol) were added to a solution of **5a** (8 g, 23 mmol) in 80 ml 2-butanone. The mixture was refluxed for 24 h and the solvent was evaporated off *in vacuo*. The residue was taken up with water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated off *in vacuo* to give a residue which was chromatographed on silica gel. Elution with CH_2Cl_2/acetone (8:2) afforded **7d** (1.9 g, 21%), mp 114°C; ^{1}H-NMR ($CDCl_3$): δ 7.72 (d, 1H, J = 7.3 Hz), 7.60 (t, 1H, J = 7.3 Hz), 7.46–7.35 (m, 4H), 7.24–7.19 (m, 2H), 4.50–4.46 (m, 2H), 4.00 (s, 2H), 3.86–3.29 (m, 2H), 3.21 (t, 1H, J = 6 Hz), 2.70 (t, 2H, J = 7.5 Hz), 2.56 (s, 3H), 1.62 (sext, 2H, J = 7.5 Hz), 0.94 (t, 3H, J = 7.5 Hz).

Further elution with CH_2Cl_2/acetone (6:4) gave **6d** (2.8 g, 31%), as an oil used without further purification for the next step; ^{1}H-NMR ($CDCl_3$): δ 7.68 (d, 1H, J = 7.3 Hz), 7.55 (t, 1H, J = 7.3 Hz), 7.43–7.28 (m, 6H), 4.35–4.27 (m, 2H), 4.24–4.15 (m, 2H), 3.80 (s, 2H), 2.69 (t, 2H, J = 7.5 Hz), 2.54 (s, 3H), 1.62 (sext, 2H, J = 7.5 Hz), 0.93 (t, 3H, J = 7.5 Hz).

2-[2-Methyl-4-oxo-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-3-yl]ethanol hydrochloride ***17***. Trimethyltin azide (2 g, 10.1 mmol) as added to a solution of **6d** (2.8 g, 7.1 mmol) in 60 ml toluene. The mixture was refluxed for 28 h under stirring and the precipitate was collected from the hot solution. The obtained crystals were taken up with an NaOH solution and the mixture was stirred for

10 min. The aqueous layer was washed with Et_2O and acidified by HCl. The crystals were collected, washed with acetone and dried to give **17** (1.2 g, 37%) as a hydrochloride, mp 240–241°C; ^{1}H-NMR (DMSO-d_6): δ 7.64 (t, 2H, J = 7.5 Hz), 7.51 (t, 2H, J = 7.5 Hz), 7.20 (d, 2H, J = 7.5 Hz), 7.05 (d, 2H, J = 7.5 Hz), 4.26–4.18 (m, 2H), 3.91 (s, 2H), 3.87–3.79 (m, 2H), 3.11 (s, 3H), 2.81 (t, 2H, J = 8 Hz), 1.76–1.65 (m, 2H), 1.02 (t, 3H, J = 8 Hz).

2-[[2-Methyl-6-n-propyl-5[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-4-yl]oxy]ethanol 21. Trimethyltin azide (2.7 g, 13.6 mmol) was added to a solution of **7d** (3.8 g, 9.6 mmol) in 80 ml toluene. The mixture was heated to reflux for 35 h. After cooling, the mixture was extracted with a KOH solution, the aqueous layer was washed with AcOEt, acidified by bubbling SO_2 and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, evaporated to dryness *in vacuo* and the residue was chromatographed on silica gel with $CHCl_3$/MeOH (8.5:1.5) to give **21** (1.7 g, 41%), mp 154–155°C; ^{1}H-NMR ($CDCl_3$): δ 8.01 (d, 1H, J = 7.3 Hz), 7.64–7.43 (m, 3H), 7.11 (br s, 4H), 4.40 (br s, 2H), 4.00 (s, 2H), 3.76–3.71 (m, 2H), 2.78 (t, 2H, J = 8 Hz), 2.59 (s, 3H), 1.75 (sext, 2H, J = 8 Hz), 1.01 (t, 3H, J = 8 Hz).

Method E

2-Methyl-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl]pyrimidin-4-(3H)-one 18. Trimethyltin azide (7.7 g, 39 mmol) was added to a solution of **5a** (10 g, 29 mmol) in 100 ml xylene. The mixture was heated to reflux for 24 h. The solid material was collected by suction from the hot solution and suspended in 50 ml THF and 50 ml toluene. Gaseous HCl was bubbled through this suspension for 20 min under vigorous stirring. Upon addition of 50 ml EtOH a precipitate appeared which was collected and dissolved in a dilute NaOH solution. This solution was washed with AcOEt, acidified by bubbling SO_2 and extracted with 100 ml $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated to half its volume *in vacuo* and 50 ml of Et_2O was added. Upon standing, crystals appeared, which were collected and dried to give 5.3 g (47%) of **32**, mp 204–206°C; ^{1}H-NMR (DMSO-d_6): δ 7.71–7.52 (m, 4H), 7.11 (d, 2H, J = 7.9 Hz), 6.97 (d, 2H, J = 7.9 Hz), 3.76 (s, 2H), 2.40 (t, 2H, J = 7.5 Hz), 2.23 (s, 3H), 1.44 (sext, 2H, J = 7.5 Hz), 0.81 (t, 3H, J = 7.5 Hz).

Method F

5-[(2'-Cyanobiphenyl-4-yl)methyl]-4-methoxy-2-methyl-6-n-propyl pyrimidine 7e. A solution of sodium (0.8 g, 34 mmol) in 10 ml MeOH was added to a solution of **8a** (12 g, 33.2 mmol) in 50 ml MeOH. The mixture was stirred for 3 h at room temperature and refluxed for 2 h. The solvent was removed *in vacuo* and the residue was taken up in water and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give **7e** (8.9 g, 75%), mp 108–109°C; ^{1}H-NMR ($CDCl_3$): δ 7.9 (d, 1H, J = 7.3 Hz), 7.54 (t, 1H, J = 7.3 Hz), 7.40–7.28 (m, 4H), 7.14 (d, 2H, J = 8 Hz), 3.94 (s, 2H), 3.90 (s, 3H), 2.70 (t, 2H, J = 8 Hz), 2.52 (s, 3H), 1.62 (sext, 2H, J = 8 Hz), 0.93 (t, 3H, J = 8 Hz).

4-Methoxy-2-methyl-6-n-propyl-5-[[2'-(1H-tetraol-5-yl)biphenyl-4-yl]methyl]pyrimidine 22. Compound **22** was prepared according to *Method E* proceeding from **7e**, and crystallized in Et_2O/acetone, yield 51%, mp 132–134°C; ^{1}H-NMR ($CDCl_3$): δ 7.90 (d, 2H, J = 7.3 Hz), 7.63–7.39 (m, 3H), 7.04–6.93 (m, 4H), 3.86 (s, 5H), 3.07 (s, 3H), 3.05 (t, 2H, J = 8 Hz), 1.55 (sext, 2H, J = 8 Hz), 0.84 (t, 3H, J = 8 Hz).

Method G

Ethyl 2-[[5-[2'-(Cyanobiphenyl-4-yl)methyl]-2-methyl-6-n-propylpyrimidin-4-yl]oxy]acetate 7f. NaH 60% (1.9 g, 47.5 mmol) was added to a solution of ethyl 2-hydroxyacetate (2 g, 47.5 mmol) in 40 ml THF. The mixture was stirred 15 min at room temperature. Compound **8a** (6.5 g, 18 mmol) in solution in 20 ml THF was added dropwise to this mixture. The mixture was then heated to reflux for 7 h and cooled to room temperature. The solvent was evaporated off *in vacuo* and the residue was taken up in Et_2O. The ethereal solution was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give **7f** (7.3 g, 94%), oil; ^{1}H-NMR ($CDCl_3$): δ 7.72 (d, 1H, J = 7.3 Hz), 7.60 (t, 1H, J = 7.3 Hz), 7.48–7.25 (m, 6H), 4.91 (s, 2H), 4.22 (q, 2H, J = 7 Hz), 4.17 (s, 2H), 2.69 (t, 2H, J = 7.5 Hz), 1.61 (sext, 2H, J = 7.5 Hz), 1.27 (t, 3H, J = 7 Hz), 0.94 (t, 3H, J = 7.5 Hz).

Ethyl 2-[2-Methyl-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-4-yl]oxyacetate 26. Compound **26** was prepared according to *Method A* from **12**, chromatography solvent CH_2Cl_2/MeOH (9:1), yield 44%, mp 149–150°C; ^{1}H-NMR ($CDCl_3$): δ 7.98 (d, 1H, J = 7.3 Hz), 7.62–7.40 (m, 3H), 7.19–7.06 (m, 4H), 4.90 (s, 2H), 4.14 (q, 2H, J = 7 Hz), 4.00 (s, 2H), 2.58 (t, 2H, J = 7.5 Hz), 2.40 (s, 3H), 1.66 (sext, 2H, J = 7.5 Hz), 1.24 (t, 3H, J = 7 Hz), 0.95 (t, 3H, J = 7.5 Hz).

Method H

2-[[5-[2'(-Cyanobiphenyl-4-yl)methyl]-2-methyl-6-n-propylpyrimidin-4-yl]mercapto]ethanol 11a. K_2CO_3 (4.6 g, 33.2 mmol) and 2-mercaptoethanol (2.3 ml, 33.2 mmol) were added to a solution of **8a** (10 g, 21.7 mmol) in 100 ml 2-butanone. The mixture was refluxed for 8 h, cooled and concentrated *in vacuo*. The residue was taken up in water, extracted with $CHCl_3$ and the organic layer was dried over $MgSO_4$. The residue, obtained after evaporation of the solvent *in vacuo*, crystallized in diisopropyl ether to give **11a** (6.7 g, 62%), mp 97°C; ^{1}H-NMR ($CDCl_3$): δ 7.73 (d, 1H, J = 7.3 Hz), 7.62 (t, 1H, J = 7.3 Hz), 7.69–7.37 (m, 4H), 7.18 (d, 2H, J = 7.5 Hz),4.70 (br s, 1H), 4.12 (s, 2H), 3.87 (t, 2H, J = 6 Hz), 3.35 (t, 2H, J = 6 Hz), 2.68–2.60 (m, 5H), 1.61 (sext, 2H, J = 7.5 Hz), 0.93 (t, 3H, J = 7.5 Hz).

2-[[2-Methyl-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-4-yl]mercapto]ethanol 34. Compound **34** was prepared according to *Method A* from **13**, chromatography solvent: $CHCl_3$/MeOH (8.5:1.5), crystallized in AcOEt, yield 45%, mp 186–189°C; ^{1}H-NMR (DMSO-d_6): δ 7.52–7.29 (m, 4H), 6.89 (s, 4H), 4.6 (br s, 1H), 3.91 (s, 2H), 3.68 (t, 2H, J = 6 Hz), 3.23 (t, 2H, J = 6 Hz), 2.47–2.38 (m, 5H), 1.43 (sext, 2H, J = 7.5 Hz), 0.75 (t, 3H, J = 7.5 Hz).

Method I

5-[2'-(Cyanobiphenyl-4-yl)methyl]-4-mercapto-2-methyl-6-n-propyl pyrimidine 10a. Thiourea (9.3 g, 122 mmol) and 0.1 ml concentrated HCl were added to a solution of **8a** (40 g, 111 mmol) in 150 ml acetone. The mixture was refluxed for 6 h, cooled to room temperature and evaporated to dryness *in vacuo*. The residue was taken up in a dilute KOH solution and washed with Et_2O. The aqueous layer was acidified by bubbling SO_2 and the crystals obtained were collected by suction, washed with water and dried to give **10a** (20.1 g, 50%), mp 204°C, ^{1}H-NMR (DMSO-d_6): δ 7.95 (d, 1H, J = 7.3 Hz), 7.80 (t, 1H, J = 7.3 Hz), 7.63–7.45 (m, 6H), 4.51 (s, 2H), 2.52 (t, 2H, J = 7.5 Hz), 2.45 (s, 3H), 1.53 (sext, 2H, J = 7.5 Hz), 0.83 (t, 3H, J = 7.5 Hz).

4-Mercapto-2-methyl-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl]methyl]pyrimidine 31. Compound **31** was prepared according to *Method A* from **12**, with $CHCl_3$/MeOH (8:2) as chromatography solvent, crystallized in AcOEt, yield 49%, mp 247–248°C; ^{1}H-NMR ($CDCl_3$): δ 7.68 (d, 1H, J = 7.3 Hz), 7.57 (t, 1H, J = 7.3 Hz), 7.50–7.40 (m, 2H), 7.16 (d, 2H, J = 7.5 Hz), 7.03 (d, 2H, J = 7.5 Hz), 4.34 (s, 2H), 2.50–2.40 (m, 5H), 1.50 (sext, 2H, J = 7.5 Hz), 0.85 (t, 3H, J = 7.5 Hz).

Method J

5-[2'-(Cyanobiphenyl-4-yl)methyl]-2-methyl-4-methylthio-6-n-propylpyrimidine 11b. K_2CO_3 (2.5 g, 18 mmol) and iodomethane (2 ml, 40 mmol) were added to a solution of **10a** (5.4 g, 15 mmol) in 100 ml acetone. The mixture was refluxed for 3 h, concentrated *in vacuo* and the residue was taken up with water and AcOEt. The organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil which crystallized in a mixture Et_2O/acetone (1:1) to afford **11b** (4.5 g, 80%), mp 134°C; ^{1}H-NMR ($CDCl_3$): δ 7.72 (d, 1H, J = 7.3 Hz), 7.62 (t, 1H, J = 7.3 Hz), 7.70–7.39 (m, 4H), 7.18 (d, 2H, J = 7.5 Hz), 4.10 (s, 2H), 2.55–2.48 (m, 5H), 2.35 (s, 3H), 1.60 (sext, 2H, J = 7.5 Hz), 0.93 (t, 3H, J = 7.5 Hz).

2-Methyl-4-methylthio-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl]methyl]pyrimidine 40. Compound **40** was prepared according to *Method A* for **12**, with $CHCl_3$/MeOH (9:1) as chromatography solvent, crystallized in Et_2O, yield 62%, mp 173–174°C; ^{1}H-NMR ($CDCl_3$): δ 7.69 (d, 1H, J = 7.3 Hz), 7.59–7.40 (m, 3H), 7.01–6.90 (m, 4H), 3.94 (s, 2H), 2.45 (s, 3H), 2.33 (s, 3H), 2.22 (t, 2H, J = 7.5 Hz), 1.56 (sext, 2H, J = 7.5 Hz), 0.82 (t, 3H, J = 7.5 Hz).

Method K

2-[[2-Methyl-6-n-propyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-4-yl]amino]ethanol 41. To a solution of **8a** (6 g, 16.6 mmol) in 100 ml xylene was added 2-aminoethanol (2.5 ml, 41 mmol) and the mixture was refluxed for 16 h. After cooling, the mixture was washed twice with 50 ml water and the organic layer was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$/MeOH (85:15) to give 2-[[5-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-6-n-propylpyrimidin-4-yl]-amino]ethanol (**9a**, 3.8 g, 60%), mp 135°C. Trimethyltin azide (2.7 g, 13.6 mmol) was added to a solution of **9a** (3.8 g, 10 mmol) in 50 ml toluene and the mixture was refluxed for 30 h. After cooling, the mixture was extracted with a NaOH solution. The aqueous layer was separated and washed with Et_2O then acidified with HCl (pH 3). The acid aqueous solution was washed with AcOEt and neutralized with $NaHCO_3$ (pH 7). The crystals obtained were collected by suction washed with water and acetone to give **41** (1.9 g, 44%), mp 149–150°C; ^{1}H-NMR (DMSO-d_6): δ 7.67–7.41 (m, 4H), 7.04 (s, 4H), 5.08 (br s, 1H), 3.93 (s, 2H), 3.62–3.49 (m, 4H), 2.52–2.42 (m, 5H), 1.59 (sext, 2H, J = 7.5 Hz), 0.91 (t, 3H, J = 7.5 Hz).

Biology

Angiotensin II receptor binding assay

Rat adrenal membranes were obtained according to a previously described method [19]. Rats were decapitated and whole adrenals were rapidly dissected from fatty tissue. They were rapidly dried and weighed. Adrenal tissues were homogenized in 100 volumes of ice-cold buffer (Tris-HCl 10 mM, saccharose 0.2 M, EDTA 1 mM, pH 7.4) with a glass-Teflon homogenizer. The homogenate was centrifuged at 3000 *g* for 10 min at 4°C. The supernatant was centrifuged again at 12 000 *g* for 13 min at 4°C. The supernatant was then ultracentrifuged in a polycarbonate tube at 102 000 *g* for 60 min at 4°C. The resulting pellet was resuspended in 50 volumes of ice-cold incubation buffer (Tris-HCl 50 mM, $MgCl_2$ 5 mM, BSA 0.25%, pH 7.2) and homogenized with the glass-teflon homogenizer. Receptor binding studies were carried out as previously described [19, 20] with slight modifications. Total reaction volume was 500 µl consisting of 100 µl membranes (25–40 µg protein), 50 µl [^{125}I]Sar^1–Ile^8–Ang II (0.2 nM, final concentration), 50 µl of various concentrations of the drugs (10^{-5} and 10^{-7} M for screening test) and 300 µl incubation buffer. Incubation time was 60 min at 25°C. Non-specific binding was measured in the presence of 1 µM (final concentration) unlabelled Ang II and was about 4–11% of total binding. The reaction was terminated by addition of 3 ml of cold washing buffer (Tris-HCl 50 mM, $MgCl_2$ 5 mM, pH 7.2), followed by rapid filtration through Whatman GF/B glass fiber filters which were washed twice with washing buffer. Each assay was performed in triplicate.

Data analysis

Competition data were analysed using the non-linear regression program Ligand [24] for an IBM-PC [22] and obtained from Elsevier-Biosoft (Cambridge, UK).

Antihypertensive effect in conscious renal artery-ligated hypertensive rats

Male CD Sprague–Dawley rats (250–270 g) were anesthetized with Ketamine (100 mg/kg, ip) and the left renal artery was completely ligated by means of a 4.0 silk suture, being careful not to damage the left kidney or left renal vein [16]. Seven days after the ligation, two procedures were performed to record the blood pressure in conscious hypertensive rats.

To estimate the oral antihypertensive potency of the Ang II receptor antagonists, groups of renal artery-ligated rats (n = 3–7 rats/dose) were dosed orally by gavage of the test compound at 10, 3 or 1 mg/kg. The SAP was measured before and 3 h after dosing by indirect tail-cuff method [17] using a sphygmomanometer (PE 300 Narco) coupled to a polygraph (Beckman R411). The change in SAP was expressed as the decrease in SAP 3 h postdose. The MAP was measured by the direct method as described by Smits [18]. Two or three days before the experiment, the animals were anesthetized as above for the surgical preparation. The left femoral artery was cannulated and the catheter was passed subcutaneously to the dorsal side of the neck and exteriorized. The catheter was connected to a Statham pressure transducer coupled to a polygraph (Beckman R411) for monitoring arterial blood pressure. The signal output was analyzed with a digital computer (Buxco Electronies, Sharon, US). The MAP was recorded before and 20 h after dosing. The change in MAP was expressed as maximal decrease in MAP observed during the experiment. When the decrease in MAP was less than –15 mm Hg, the drug was considered to be without effect.

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