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**Original paper**

# Synthesis and biological evaluation of 2-alkoxyphenyl-6-(substituted phenyl)-1,3,4-thiadiazole-[3,2-*a*]-*s*-triazin-5,7-diones and 1-(alkyl and substituted phenyl)-3-[5-alkoxyphenyl-1,3,4-thia- and oxadiazol-2-yl]-ureas

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**Summary** — As an extension of a previous work, in which a series of 2-alkoxyphenyl-6-phenyl-1,3,4-thiadiazole-[3,2-*a*]-*s*-triazin-5,7-diones were synthesized and found to possess analgesic activity, a new series of analogues substituted in the 6-phenyl ring has been prepared and tested with the aim of examining the variations induced by phenyl substituents in biological activity, and thus obtaining useful information about the structure–activity relationship.

Together with the series of ureas precursor to title diones, the corresponding series containing a 5-alkoxyphenyloxadiazole nucleus in place of the thiadiazole moiety, and some alkyl ureas were also prepared and tested for biological activity.

**Résumé** — **Synthèse et évaluation biologique d'alkyloxyphényl-2 (phényl substitué)-6 thiadiazole-1,3,4 [3,2-*a*]-*s*-triazinediones-5,7 et d'(alkyl et phényl substitué)-1 [alkyloxyphényl-5 (thia- et oxadiazolyl-2)-1,3,4]-3 urées.** *Précédemment, nous avons synthétisé une série d'alkyloxyphényl-2 phényl-6 thiadiazole-1,3,4 [3,2-a]-s-triazinediones-5,7 dont plusieurs d'entre elles ont présenté une activité analgésique. Dans la présente recherche, nous avons préparé et testé une nouvelle série d'analogues substitués en 6 sur le cycle phényle, dans le but d'examiner les variations induites par les substituants du phényle sur l'activité biologique et obtenir des indications valables sur la relation structure–activité.*

*Avec les séries des urées précurseurs des diones du titre, on a aussi préparé et testé l'activité biologique des séries correspondantes contenant un groupement alkyloxyphényl-5-oxadiazolique au lieu de la partie thiadiazolique de quelques alkylurées.*

**ureas / thiadiazoles / oxadiazoles / 1,3,4-thiadiazole-[3,2-*a*]-*s*-triazin-5,7-diones / anti-inflammatory activity / analgesic activity / antibacterial activity / antifungal activity**

## Introduction

In an earlier work [1] 2 series of 2-alkoxyphenyl-6-phenyl-1,3,4-thia- and oxadiazole-[3,2-*a*]-*s*-triazin-5,7-diones **IV**, along with phenylureas **II** and acetamides **III** were prepared starting from the corresponding previously described 2-amino-5-alkoxyphenyl-1,3,4-thia- and oxadiazoles **I** [2], in which the biological importance of the alkoxyphenyl 5-substituent has already been widely discussed [3, 4] (Scheme 1).

In the same work, pharmacological tests performed on bicyclic compounds **IV** showed, for several thiadiazole derivatives, good analgesic activity, which was accompanied in many by a more modest anti-inflammatory activity.

On the basis of these results, we decided to prepare, in a first application, a new series of thiadiazole-[3,2-*a*]-*s*-triazin-5,7-diones **IV** substituted in the 6-phenyl ring (compounds **1**–**15**, Table I) and examine the probable effect of phenyl substituents on the biological activity. Furthermore, since one of the 2 alternative synthetic approaches to compounds **IV** required the preparation of thiadiazolylureas **II** as intermediates–and the thiadiazole nucleus is known to be responsible for antiparasitic [5, 7], antibacterial, and antimycotic activities [2, 8, 9] in many structures–we considered it interesting to synthesize and test a series of ureas **II**, including a number of terms (com-

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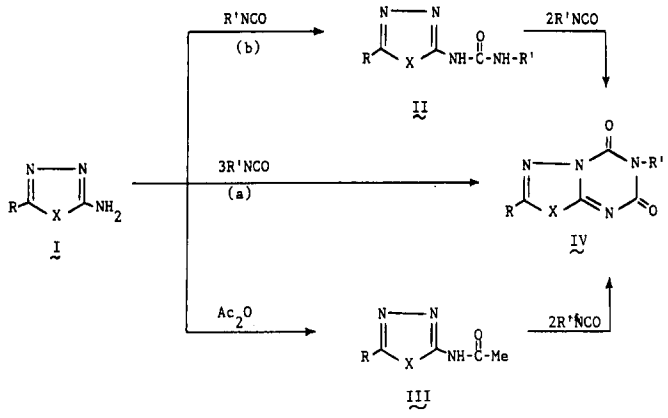


**Scheme 1.**

X = O; S. R = 3,4,5-trimethoxyphenyl; 3,4-dioxymethylenephenyl; 3,5-dimethoxy-4-ethoxyphenyl. $R^1$ = phenyl; substituted phenyl; alkyl.

pounds **19–51**, Table II) exceeding the precursors of the planned diones **1–15**. At the same time we synthesized and tested the corresponding series of analogues (compounds **55–87**) containing the oxadiazole nucleus as N-substituent in place of that of thiadiazole, in order to examine the effect of oxygen–sulfur isosteric displacement, besides that of phenyl substituents.

The introduction of substituents into the phenyl ring of the 2 classes of systems **II** and **IV** was accomplished with the principal aim of achieving variations in the lipophilicity of the new molecules, taking into account their known influence on the biological activity [10–12].

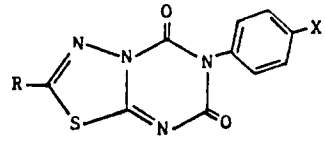
On the other hand, it is also known that the introduction of substituent can condition the attack of a molecule on the receptor by modulating the molecule's polarity. On the basis of this latter consideration, ethyl- and cyclohexyl-ureas (compounds **46–51**, X=O, and **82–87**, X=S) were also prepared.

All the synthesized compounds were subjected to biological screening; particularly, bicyclic derivatives **IV** were tested mainly for their anti-inflammatory and analgesic activities, and ureas **II** for their antibacterial and antimycotic activities.

## Chemistry

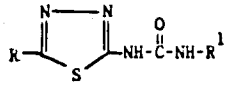
Synthetic approaches for the title compounds are outlined

**Table I.** 2-Alkoxyphenyl-6-(substituted phenyl)-1,3,4-thiadiazole-[3,2-*a*]-*s*-triazin-5,7-diones **1–15**.



| Compound No. | R | X | Yield (%) | mp (°C) | Cryst. Solvent | IR (KBr) C=O | $cm^{-1}$ |
|---|---|---|---|---|---|---|---|
| **1** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | Cl | 80 | 274–75 | DMF/EtOH | 1750 | 1690 |
| **2** | 3,4-$(OCH_2O)$-$C_6H_3$ | Cl | 70 | 280–81 | DMF | 1745 | 1690 |
| **3** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | Cl | 70 | 277–78 | DMF | 1750 | 1685 |
| **4** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | F | 70 | 275–77 | DMF | 1760 | 1690 |
| **5** | 3,4-$(OCH_2O)$-$C_6H_3$ | F | 70 | 282–83 | DMF | 1765 | 1690 |
| **6** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | F | 75 | 279–81 | DMF/EtOH | 1765 | 1695 |
| **7** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | $CH_3$ | 80 | 266–67 | DMF | 1755 | 1695 |
| **8** | 3,4-$(OCH_2O)$-$C_6H_3$ | $CH_3$ | 70 | 287–88 | DMF | 1760 | 1690 |
| **9** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | $CH_3$ | 75 | 258–59 | DMF/EtOH | 1740 | 1690 |
| **10** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | $OCH_3$ | 75 | 280–81 | DMF | 1740 | 1700 |
| **11** | 3,4-$(OCH_2O)$-$C_6H_3$ | $OCH_3$ | 75 | 283–84 | DMF | 1750 | 1705 |
| **12** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | $OCH_3$ | 70 | 273–75 | DMF | 1745 | 1700 |
| **13** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | $NO_2$ | 60 | 298–99 | EtOH | 1760 | 1700 |
| **14** | 3,4-$(OCH_2O)$-$C_6H_3$ | $NO_2$ | 55 | 292–93 | DMF/EtOH | 1755 | 1690 |
| **15** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | $NO_2$ | 60 | 295–96 | DMF-EtOH | 1745 | 1690 |

**Table II.** 1-(Alkyl and substituted phenyl)-3-(5-alkoxyphenyl-1,3,4-thiadiazol-2-yl)-ureas **19**–**51**.



| Compound No. | R | $R^1$ | Yields | Cryst. | mp | IR (KBr) | $cm^{-1}$ |
|---|---|---|---|---|---|---|---|
| | | | (%) | solvent | (°C) | NH | C=O |
| **19** | $3,4,5\text{-}(OCH_3)_3\text{-}C_6H_2$ | $4\text{-}Cl\text{-}C_6H_4$ | 75 | DMF | 305–306 | 3370 | 1725 |
| **20** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $4\text{-}Cl\text{-}C_6H_4$ | 80 | DMF | >340 | 3385 | 1695 |
| **21** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $4\text{-}Cl\text{-}C_6H_4$ | 75 | DMF / EtOH | 317–18 | 3320 | 1670 |
| **22** | $3,4,5\text{-}(OCH_3)_3\text{-}C_6H_2$ | $3\text{-}Cl\text{-}C_6H_4$ | 75 | DMF | 316–18 | 3370 | 1720 |
| **23** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $3\text{-}Cl\text{-}C_6H_4$ | 80 | DMF | >340 | 3380 | 1720 |
| **24** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $3\text{-}Cl\text{-}C_6H_4$ | 80 | DMF | 328–30 | 3370 | 1725 |
| **25** | $3,4,5,\text{-}(OCH_3)_3\text{-}C_6H_2$ | $3,4\text{-}Cl_2\text{-}C_6H_3$ | 80 | DMF | 308–309 | 3360 | 1720 |
| **26** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $3,4\text{-}Cl_2\text{-}C_6H_3$ | 75 | DMF | >340 | 3370 | 1725 |
| **27** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $3,4\text{-}Cl_2\text{-}C_6H_3$ | 80 | DMF | 329–30 | 3370 | 1720 |
| **28** | $3,4,5,\text{-}(OCH_3)_3\text{-}C_6H_2$ | $4\text{-}F\text{-}C_6H_4$ | 70 | DMF / EtOH | 324–25 | 3390 | 1725 |
| **29** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $4\text{-}F\text{-}C_6H_3$ | 75 | DMF | >340 | 3370 | 1720 |
| **30** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $4\text{-}F\text{-}C_6H_4$ | 75 | DMF / EtOH | 332–33 | 3380 | 1725 |
| **31** | $3,4,5,\text{-}(OCH_3)_3\text{-}C_6H_2$ | $3\text{-}F\text{-}C_6H_4$ | 75 | DMF | 314–15 | 3380 | 1725 |
| **32** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $3\text{-}F\text{-}C_6H_4$ | 70 | DMF | >340 | 3380 | 1720 |
| **33** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $3\text{-}F\text{-}C_6H_4$ | 70 | DMF | 324–25 | 3390 | 1725 |
| **34** | $3,4,5\text{-}(OCH_3)_3\text{-}C_6H_2$ | $4\text{-}CH_3\text{-}C_6H_4$ | 75 | DMF / EtOH | 312–13 | 3390 | 1730 |
| **35** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $4\text{-}CH_3\text{-}C_6H_4$ | 70 | DMF | >340 | 3370 | 1720 |
| **36** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $4\text{-}CH_3\text{-}C_6H_4$ | 75 | DMF / EtOH | 330–31 | 3380 | 1710 |
| **37** | $3,4,5\text{-}(OCH_3)_3\text{-}C_6H_2$ | $4\text{-}OCH_3\text{-}C_6H_4$ | 70 | DMF | 302–302 | 3390 | 1720 |
| **38** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $4\text{-}OCH_3\text{-}C_6H_4$ | 70 | DMF | >340 | 3380 | 1700 |
| **39** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $4\text{-}OCH_3\text{-}C_6H_4$ | 70 | DMF | 317–18 | 3390 | 1725 |
| **40** | $3,4,5\text{-}(OCH_3)_3\text{-}C_6H_2$ | $3\text{-}OCH_3\text{-}C_6H_4$ | 75 | DMF | 312–13 | 3380 | 1725 |
| **41** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $3\text{-}OCH_3\text{-}C_6H_4$ | 75 | DMF | >340 | 3360 | 1710 |
| **42** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $3\text{-}OCH_3\text{-}C_6H_4$ | 75 | DMF | 306–307 | 3320 | 1720 |
| **43** | $3,4,5,\text{-}(OCH_3)_3\text{-}C_6H_2$ | $4\text{-}NO_2\text{-}C_6H_4$ | 80 | DMF / EtOH | 317–18 | 3360 | 1735 |
| **44** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $4\text{-}NO_2\text{-}C_6H_4$ | 75 | DMF | 319–20 | 3370 | 1725 |
| **45** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $4\text{-}NO_2\text{-}C_6H_2$ | 75 | DMF | 322 | 3370 | 1730 |
| **46** | $3,4,5,\text{-}(OCH_3)_3\text{-}C_6H_2$ | $C_2H_5$ | 70 | DMF / EtOH | 302–303 | 3380 | 1690 |
| **47** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $C_2H_5$ | 75 | AcOH | >340 | 3390 | 1695 |
| **48** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $C_2H_5$ | 80 | AcOH | 332–33 | 3390 | 1690 |
| **49** | $3,4,5\text{-}(OCH_3)_3\text{-}C_6H_2$ | $C_6H_{11}$ | 75 | AcOH | 294–95 | 3380 | 1720 |
| **50** | $3,4\text{-}(OCH_2O)\text{-}C_6H_3$ | $C_6H_{11}$ | 70 | AcOH | >340 | 3390 | 1685 |
| **51** | $3,5\text{-}(OCH_3)_2\text{-}4\text{-}OC_2H_5\text{-}C_6H_2$ | $C_6H_{11}$ | 70 | AcOH | 300–301 | 3390 | 1705 |

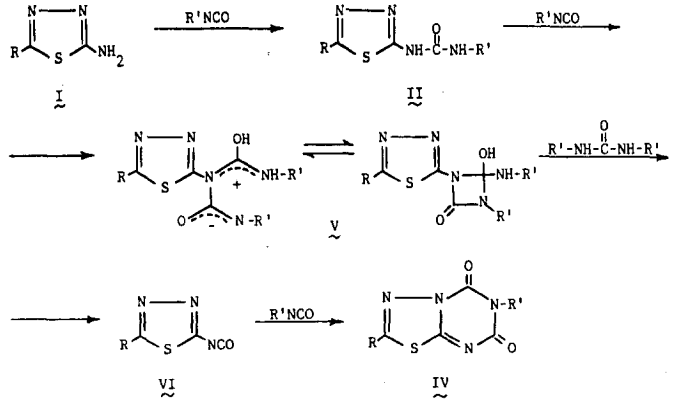
in pathways a and b of Scheme 1. The bicyclic structures **1–6** and **13–15** were obtained by refluxing a pyridine solution of aminoheterocycles **I** (X=S) and a 3-fold excess of the appropriate isocyanate or a pyridine solution of corresponding ureas **II** and a 2-fold excess of isocyanate until reactants were no longer monitored by TLC, according to 2 of the approaches previously used by us [1] and others for triazinediones condensed with analogous or different heterocycles [13–16]. Owing to the lower reactivity of *p*-methoxy- and *p*-methylphenylisocyanate the synthesis of derivatives **7–12** necessitated heating reaction mixtures in a sealed tube at 150°C for 12 h. Probably because of steric impediments, *meta*-substituted phenylisocyanates reacted to a very low extent (2–5%) in all the attempted reaction conditions whether with aminoheterocycles or with ureas, thus discouraging any further attempt to prepare 6-(*m*-substituted phenyl) derivatives **IV**.

Alkylisocyanates proved to be nonreactive, as was found for their reactions with amino-oxazoles and corresponding acetamide and urea derivatives [14].

Ureas **II** (compounds **19–87**) were prepared by reaction of aminoheterocycles **I** with the appropriate isocyanate in xylene as solvent, which afforded better yields than pyridine as previously employed [1]. Anhydrous reagents and solvents were necessary, otherwise symmetrical ureas were formed as by-products deriving from the hydrolysis of isocyanates. These symmetrical ureas were, however, produced in the reactions for condensed systems **IV** from fragmentation of the initially formed dipolar adducts **V**, which also generated thiadiazole isocyanates **VI** affording bicyclic structures **IV** through a [2+4] cycloaddition to the arylisocyanate present in excess, according to Scheme 2 [17].

Their separation proved to be difficult and considerably decreased the isolated yields of the desired compound in some cases.

The structures of synthesized compounds were confirmed by elemental analysis and IR and mass spectra. Bicyclic systems **1–15** showed 2 clearly distinct strong IR absorption bands in the ranges 1685–1705 $cm^{-1}$ and 1740–1765 $cm^{-1}$ for the 2 carbonyl groups present in 5- and 7-positions, while ureas **19–87** displayed 2 characteristic bands in the ranges 1690–1730 $cm^{-1}$ and 3270–3380 $cm^{-1}$ associated with the stretchings of C=O and N–H bonds, respectively.



**Scheme 2.**

Mass spectra of triazinediones **1–15** were characterized by molecular ions of high intensity, isocyanate radical ions (base peaks) deriving from a 1,4-retrocycloaddition fragmentative process, and ions generated from these latter by loss of carbon monoxide. Besides these, ions arising from the ring fission of the fragment incorporating the heterocyclic nucleus were also observed. Electron impact of thia- and oxadiazolylureas **19–51** and **55–87**, respectively, gave fragile molecular ions which underwent a primary fragmentation process leading to ions, found as base peaks in the spectra, corresponding to 2-amino-5-alkoxyphenyl heterocycles. From these a series of ions were originated according to the known fission of 2-amino-5-aryl-1,3,4-thiadiazoles [18, 19].

## Results and Discussion

Pharmacological results for condensed triazinedione derivatives **1–15** are given in Table IV, and are discussed below in comparison with their analogues with 6-unsubstituted phenyl ring **16–18** described previously [1].

Acute toxicity tests showed that none of the screened compounds produces lethal effects up to the maximum administered dose of 1000 mg/kg/*p.o.* and 500 mg/kg/i.p., while the phenylbutazone $LD_{50}$ was found to be 620 mg/kg/*p.o.* and 280 mg/kg/i.p., respectively.

The synthesized compounds did not induce any significant behaviour modification in Irwin's test.

At the administered dose (10 mg/kg/*p.o.*), the phenylquinone writhing test indicated for some terms (compounds **1, 4, 7, 10** and **11**) a higher analgesic activity than that of phenylbutazone, but lower in any case than that of analogues containing an unsubstituted phenyl ring.

From an examination of data reported in Table IV, it can be noted that: (a) the introduction of electron-withdrawing (Cl, F, $NO_2$) or electron-donor substituents (Me, MeO) in the *para*-position of the phenyl ring produces a decrease of activity which, however, remains at levels comparable with those of phenylbutazone in the case of the most active derivatives. Such a reduction in activity appears to follow the order Me>$NO_2$>Cl>F>MeO when the found values are considered; (b) derivatives 2-linked with the 3,4,5-trimethoxyphenyl group showed a greater activity than terms with 3,4-dioxymethylenephenyl and 3,5-dimethoxy-4-ethoxyphenyl groups, confirming the observations reported in the preceding paper [1].

In the carrageenin paw edema test all compounds at 100 mg/kg/*p.o.* were weakly active or completely devoid of anti-inflammatory activity.

In the acetic acid-induced peritonitis assay, at the dose of 100 mg/kg/*p.o.* only some terms (compounds **6**, **7**, and **10**) showed an anti-exudative effect, which could be comparable to that of phenylbutazone.

With regard to antipyretic activity and effects induced on the CNS, the tested compounds, as well as the corresponding derivatives with a 6-unsubstituted phenyl ring, were not found to be associated with any activity.

The test conducted to evaluate ulcerogenic effects indicated that animals treated with a dose of 400 mg/kg/*p.o.* (200 mg/kg × 2) did not show any gastric lesion, as was

observed with unsubstituted phenyl parent compounds, while phenylbutazone caused lesions in all tested animals at a total dose of 200 mg/kg.

Microbiological screenings were performed on thia- and oxadiazolylureas (compounds **19–51** and **55–87**, respectively) and on the *N*-unsubstituted phenyl derivatives (compounds **52–54**), which were not investigated in our previous work [1] with regard to their biological activity.

Results showed that many thiadiazolylureas proved to possess antibacterial and antifungal activities, showing *MICs* ranging between 6.25 and 100 $\mu$g/ml. Only *MIC* value of more active terms are given in Table V, in which *MICs* of more than 50 $\mu$g/ml incidentally have not been reported.

With reference to the influence of substituents present in the 2 *N*-linked moieties on the microbiological activity, it would seem that the alkoxyphenyl group in the thiadiazole nucleus does significantly affect molecular activity. Ureas with an *N*-unsubstituted phenyl ring (compounds **52–54**) are associated with *MICs* ranging between 12.5 and 50 $\mu$g/ml and appear to show greater antibacterial activity but lower antimycotic activity. Among *N*-substituted phenylureas, the terms containing a chlorine atom in the *para*-position (compounds **19–21** and **25–27**) would appear to induce higher antibacterial activity; their *MIC* values, however, are not far from those found for unsubstituted phenyl derivatives **52–54**.

By contrast, introduction of a methoxy- and a methyl-group considerably lowers the activity. Finaly, *N*-alkyl ureas were also found to be remarkably less active: compounds **46–51** showed *MICs* higher than 50–100 $\mu$g/ml.

Oxadiazolylureas **82–87** proved to be inactive at concentrations of 200 $\mu$g/ml, the highest used.

These latter results would suggest that the activity of thiadiazolylureas **19–51**–which, however, for all terms appears much lower than that showed by drugs commonly employed in therapy (*i.e.* miconazole, Table V) – may be ascribed with very high probability to the potential thiourea moiety -S-C$\begin{matrix}-NH- \\ =N-\end{matrix}$ present in these structures, in further confirmation of the bioequivalence between thiazole and thiadiazole nuclei.

In view of these results, it would be interesting to extend investigations to analogous thiadiazolylthiourea derivatives where the presence of a real thiourea group should increase biological activity. We intend to test this hypothesis in a forthcoming work.

## Experimental protocols

### Chemistry

Melting points were determined by a Büchi model 510 apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer model 281 spectrophotometer using potassium bromide wafers. Mass spectra were obtained with an LKB 9000 S instrument. Elemental analyses (C,H,N) were performed on a Carlo Erba model 1106 elemental analyzer and were within ±0.4% of theoretical values.

The purity of compounds and the end of reactions were checked by TLC, which was performed on Merck silica gel 60-$F_{254}$ precoated aluminium plates, using cyclohexane–ethyl acetate mixtures (10:90, 30:70 or 50:50 vol/vol) as eluents.

Identification of samples obtained from different experiments was confirmed by mixed melting points and superimposable IR spectra.

### Materials

2-Amino-5-alkoxyphenyl-1,3,4-thia- and oxadiazoles **I** were prepared as previously described [2]. Aryl- and alkylisocyanates were commercially available preparations. Pyridine and xylene were purified and dried according to reported procedures [19].

*2-Alkoxyphenyl-6-(substituted phenyl)-1,3,4-thiadiazole-[3,2-a]-s-triazin-5,7-diones (**1–15**)*

A solution of aminothiadiazole **I** (X=S) (0.01 mol) and substituted phenylisocyanates (0.03 mol) in anhydrous pyridine (30 ml) was heated under reflux and a nitrogen atmosphere, until isocyanate was no longer monitored by TLC. Evaporation *in vacuo* gave a dark brown solid, which was dissolved in hot acetic acid (100 ml) and treated with charcoal. After filtration and cooling, bicyclic dione precipitated as a pale powder, which was filtered off and recrystallized from a suitable solvent until symmetrical urea was removed. For terms **7–12**, a solution of parent compound **I** (0.01 mol) and isocyanate (0.03 mol) in anhydrous pyridine (30 ml) was heated at 150°C in a deaerated sealed tube for 12 h and the resulting reaction mixture was worked up as above.

Alternatively, structures **1–15** were obtained starting from corresponding ureas **II** and a 2-fold excess of isocyanate under the same experimental conditions used for aminothiadiazoles **I** (see Scheme I).

Melting points, crystallization solvents, yields, and IR data of derivatives **1–15** are given in Table I.

*1-(Substituted phenyl)-3-(5-alkoxyphenyl-1,3,4-thias- and oxadiazol-2-yl)-ureas (**19–87**)*

A solution of aminothia- and oxadiazole **I** (0.01 mol) and substituted phenylisocyanate (0.012 mol) in anhydrous xylene (20 ml) was heated under reflux until isocyanate was no longer monitored by TLC. Evaporation *in vacuo* gave a solid which was crystallized from a suitable solvent. In some cases several recrystallizations were required to remove the symmetrical urea also formed as a reaction product.

Melting points, crystallization solvents, yields, and IR data for ureas **19–87** are reported in Tables II and III.

### Pharmacology

Compounds **1–15** were tested for anti-inflammatory, antipyretic, ulcerogenic, and principally analgesic activities. In addition, behavioral and potential CNS effects were investigated. Phenylbutazone (PBZ) was included as a reference drug under the same experimental conditons. All compounds were administered orally or intraperitoneally as a 0.5% methylcellulose aqueous suspension.

Tests were performed on Swiss male mice of 28–30 g body wt and Sprague–Dawley male rats of 130–150 g body wt. The animals were starved for about 15 h before administration.

Statistical analysis was performed by Student's *t*-test *versus* controls. The level of significance was set at $P<0.05$.

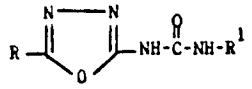
*Behavioral effects and acute toxicity*

Irwin's multidimensional screening-evaluative procedure [20] was used on groups of 5 mice. The compounds were administered at 3 dosage levels orally (500–750–1000 mg/kg) or intraperitoneally (125–250–500 mg/kg). The animals were kept under observation for 6 h after treatment. Orientational acute toxicity ($LD_{50}$) was evaluated during the 7 d following treatment.

*Analgesic activity (phenylquinone writhing test)*

Tests were performed according to the technique of Berkowitz *et al.* [21]. Groups of 6 mice were injected i.p. with 0.25 ml of a 0.02% hydroalcoholic solution of phenylquinone 60 min after oral administration of test compounds. The writhing response frequency was counted in each animal for 5 min (between min 5 and min 10) after injection of the irritant. Analgesic effect was expressed as percent protection in comparison with controls (Table IV).

**Table III.** 1-(Alkyl and substituted phenyl)-3-(5-alkoxyphenyl-1,3,4-oxadiazol-2-yl)-ureas **55–87**.



| Compound No. | R | $R^1$ | Yields (%) | Cryst. solvent | mp (°C) | IR (KBr) NH | IR (KBr) C=O ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|
| **55** | 3,4,5,-$(OCH_3)_3$-$C_6H_2$ | 4-Cl-$C_6H_4$ | 80 | EtOH | 222–23 | 3290 | 1700 |
| **56** | 3,4-$(OCH_2O)$-$C_6H_3$ | 4-Cl-$C_6H_4$ | 75 | DMF | 241–42 | 3260 | 1720 |
| **57** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 4-Cl-$C_6H_4$ | 80 | DMF / EtOH | 234–35 | 3285 | 1695 |
| **58** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 3-Cl-$C_6H_4$ | 75 | EtOH | 200–201 | 3225 | 1710 |
| **59** | 3,4,-$(OCH_2O)$-$C_6H_3$ | 3-Cl-$C_6H_4$ | 70 | DMF | 246–47 | 3225 | 1710 |
| **60** | 3,5-$(OCH_3)_2$-4-$OCH_2H_5$-$C_6H_2$ | 3-Cl-$C_6H_4$ | 60 | DMF | 194–95 | 3230 | 1700 |
| **61** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 3,4-$Cl_2$-$C_6H_3$ | 85 | DMF | 214–15 | 3260 | 1695 |
| **62** | 3,4-$(OCH_2O)$-$C_6H_3$ | 3,4-$Cl_2$-$C_6H_3$ | 65 | DMF | 234–35 | 3270 | 1695 |
| **63** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 3,4-$Cl_2$-$C_6H_3$ | 70 | EtOH | 215–16 | 3260 | 1690 |
| **64** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 4-F-$C_6H_4$ | 80 | DMF | 237–38 | 3290 | 1710 |
| **65** | 3,4-$(OCH_2O)$-$C_6H_3$ | 4-F-$C_6H_4$ | 80 | DMF | 228–30 | 3285 | 1705 |
| **66** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 4-F-$C_6H_4$ | 75 | DMF | 223–24 | 3290 | 1710 |
| **67** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 3-F-$C_6H_4$ | 70 | AcOH | 225–27 | 3280 | 1690 |
| **68** | 3,4-$(OCH_2O)$-$C_6H_3$ | 3-F-$C_6H_4$ | 70 | AcOH | 233–34 | 3280 | 1690 |
| **69** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 3-F-$C_6H_4$ | 75 | AcOH | 194–96 | 3280 | 1695 |
| **70** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 4-$CH_3$-$C_6H_4$ | 70 | EtOH | 215–16 | 3280 | 1690 |
| **71** | 3,4-$(OCH_2O)$-$C_6H_3$ | 4-$CH_3$-$C_6H_4$ | 75 | EtOH | 205–206 | 3280 | 1710 |
| **72** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 4-$CH_3$-$C_6H_4$ | 70 | EtOH | 185–86 | 3275 | 1690 |
| **73** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 4-$OCH_3$-$C_6H_4$ | 70 | EtOH | 220–21 | 3280 | 1700 |
| **74** | 3,4-$(OCH_2O)$-$C_6H_3$ | 4-$OCH_3$-$C_6H_4$ | 70 | EtOH | 229–30 | 3275 | 1690 |
| **75** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 4-$OCH_3$-$C_6H_4$ | 65 | EtOH | 202–203 | 3280 | 1690 |
| **76** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | 3-$OCH_3$-$C_6H_4$ | 75 | EtOH | 215–16 | 3280 | 1690 |
| **77** | 3,4-$(OCH_2O)$-$C_6H_3$ | 3-$OCH_3$-$C_6H_4$ | 70 | EtOH | 205–206 | 3280 | 1710 |
| **78** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 3-$OCH_3$-$C_6H_4$ | 75 | EtOH | 185–87 | 3275 | 1690 |
| **79** | 3,4,5,-$(OCH_3)_3$-$C_6H_2$ | 4-$NO_2$-$C_6H_4$ | 70 | AcOH | 255–56 | 3290 | 1700 |
| **80** | 3,4-$(OCH_2O)$-$C_6H_3$ | 4-$NO_2$-$C_6H_4$ | 65 | AcOH | 250–52 | 3300 | 1680 |
| **81** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | 4-$NO_2$-$C_6H_4$ | 70 | AcOH | 280–81 | 3290 | 1690 |
| **82** | 3,4,5-$(OCH_3)_3$-$C_6H_2$ | $C_2H_5$ | 75 | EtOH | 225–26 | 3270 | 1730 |
| **83** | 3,4-$(OCH_2O)$-$C_6H_3$ | $C_2H_5$ | 60 | EtOH | 233–34 | 3280 | 1720 |
| **84** | 3,5-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | $C_2H_5$ | 65 | EtOH | 216–17 | 3270 | 1725 |
| **85** | 3,4,5,-$(OCH_3)_3$-$C_6H_2$ | $C_6H_{11}$ | 80 | EtOH | 209–10 | 3305 | 1690 |
| **86** | 3,4-$(OCH_2O)$-$C_6H_3$ | $C_6H_{11}$ | 75 | EtOH | 219–21 | 3295 | 1675 |
| **87** | 3,4-$(OCH_3)_2$-4-$OC_2H_5$-$C_6H_2$ | $C_6H_{11}$ | 75 | EtOH | 184–85 | 3315 | 1690 |

**Table IV.** Pharmacological data of 2-alkoxyphenyl-6-(substituted phenyl)-1,3,4-thiadiazole-[3,2-*a*]-*s*-triazin-5,7-diones **1–15**.

| Compound No. | Anti-inflammatory activity | | Analgesic activity |
|---|---|---|---|
| | inhibition % carrageenin edema (3 h) 100 mg / kg *p.o.* | inhibition % acetic acid peritonitis 100 mg / kg *p.o.* | inhibition % writhing test 100 mg / kg *p.o.* |
| **1** | 18 | 18 | 38* |
| **2** | 16 | 28* | 33* |
| **3** | 13 | 14 | 27* |
| **4** | 8 | 19 | 36* |
| **5** | 7 | 18 | 31* |
| **6** | 4 | 10 | 22* |
| **7** | 16 | 36* | 45* |
| **8** | 9 | 17 | 31* |
| **9** | 6 | 13 | 28* |
| **10** | 11 | 29* | 36* |
| **11** | 11 | 19 | 35* |
| **12** | 5 | 12 | 26* |
| **13** | 25* | 18 | 36* |
| **14** | 22 | 14 | 33* |
| **15** | 20 | 13 | 28* |
| **16**[a] | 37* | 10 | 59* |
| **17**[a] | 30* | 20 | 59* |
| **18**[a] | 29* | 13 | 53* |
| Phenylbutazone | 58* | 34* | 34* |

*$P<0.05$, Student's *t*-test *versus* controls.
[a]These compounds have been reported previously [1].

*Anti-inflammatory activity*
*Carrageenin-induced paw edema [22].* Groups of 5 rats were used. Thirty min after the oral administration of test drugs, 0.1 ml of a 1% carrageenin solution was injected into the subplantar tissue of the right hindpaw and volume was measured by a mercury plethysmometer. The increase in volume of the paw 3 h after the injection of carrageenin was adopted as a measure of edema. Swelling in treated animals was calculated as percent inhibition in comparison with controls (Table IV).
*Acetic acid peritonitis [23].* Groups of 5 rats were given i.p. 10 ml / kg of a 5% acetic acid solution, 60 min after oral administration of the test compounds. Thirty min later, rats were killed in ether and the peritoneal exudate was collected and measured. The anti-exudative response was expressed as the percent exudate volume reduction compared with controls (Table IV).

*Antipyretic activity*
Tests were performed on rats according to the method of Winder *et al.* [24]. Groups of 5 animals were injected s.c. with a 15% aqueous yeast suspension in a volume of 10 ml / kg. Sixteen h later, rectal temperature was measured by a Medeor Thermorapid apparatus. The compounds were given orally and the temperature was measured hourly for 4 h, by recording the differences from the initial values.

*Ulcerogenic activity*
Groups of 4 rats were used. All compounds were given orally to animals fasted for 24 h and after 2 h the treatment was repeated [25]. Six h after the first dosing each rat was sacrificed by ether inhalation; the stomach was removed, opened along the greater curvature, and examined with a dissecting microscope for the presence of gastric ulcers.

*Effects on central nervous system*
The central effects of test compounds were investigated in mice (4 animals / group) by various standard tests. Drugs were administered i.p. at 200 mg / kg. The following tests were carried out 30 min after treatment.
*Duration of barbiturate sleep.* Pretreated mice were injected i.p. with barbital (160 mg / kg) and sleep time was measured. The recovery of righting reflex was used as the endpoint of sleep.
*Anticonvulsant activity.* Mice were given an aqueous solution of pentylenetetrazole (100 mg / kg) s.c. The antagonism of pentylenetetrazole-induced clonic convulsions was evaluated for a period of 20 min.
*Antagonism of reserpine-induced ptosis and hypothermia.* Mice received reserpine (2.5 mg / kg i.p.). Ptosis scores were evaluated after 60 min, as described by Rubin *et al.* [26], and hypothermia after 120 min.
*Catalepsy.* Pretreated mice were placed so that their forepaws rested

**Table V.** *In vitro** antibacterial and antimycotic activities of some 1-(alkyl and substituted phenyl)-3-(5-alkoxyphenyl-1,3,4-thiadiazol-2-yl)-ureas.

| Compound No. | Bacteria | | Mycetes | | | |
|---|---|---|---|---|---|---|
| | *Escherichia coli* | *Staphylococcus aureus* | *Candida albicans* | *Candida krusei* | *Candida parapsilosis* | *Torulopsis glabrata* |
| **19** | 6.25 | 6.25 | 25 | 25 | 25 | 25 |
| **20** | 6.25 | 6.25 | 25 | 50 | 25 | 25 |
| **21** | 12.5 | 12.5 | 50 | 50 | 50 | 50 |
| **22** | 12.5 | 12.5 | 50 | 50 | 50 | 50 |
| **23** | 12.5 | 12.5 | 25 | 50 | 50 | 50 |
| **24** | 12.5 | 12.5 | 50 | 50 | 50 | 50 |
| **25** | 6.25 | 6.25 | 25 | 25 | 25 | 25 |
| **26** | 6.25 | 6.25 | 12.5 | 12.5 | 25 | 25 |
| **27** | 6.25 | 6.25 | 25 | 25 | 25 | 25 |
| **28** | 12.5 | 12.5 | 25 | 50 | 50 | 25 |
| **29** | 12.5 | 12.5 | 12.5 | 12.5 | 25 | 50 |
| **30** | 12.5 | 12.5 | 50 | 50 | 50 | 50 |
| **31** | 12.5 | 12.5 | 25 | 25 | 50 | 50 |
| **32** | 12.5 | 12.5 | 12.5 | 12.5 | 25 | 25 |
| **33** | 12.5 | 25 | 25 | 25 | 25 | 50 |
| **40** | 50 | 50 | 6.25 | 6.25 | 6.25 | 12.5 |
| **41** | 25 | 25 | 6.25 | 6.25 | 6.25 | 12.5 |
| **42** | 50 | 50 | 6.25 | 6.25 | 6.25 | 12.5 |
| **52**[a] | 12.5 | 12.5 | 25 | 25 | 50 | 50 |
| **53**[a] | 12.5 | 12.5 | 25 | 25 | 50 | 50 |
| **54**[a] | 12.5 | 12.5 | 25 | 50 | 25 | 25 |
| Miconazole | 12.5 | 12.5 | 1.56 | 0.39 | 0.39 | 3.12 |

**MICs* are expressed in micrograms per milliliter.
[a]These compounds have been reported previously [1].
Compounds **43**–**51** and **55**–**87** showed an *MIC* >100 μg/ml.

on a 5-cm high pedestal and the number of seconds, to a maximum of 30 s, during which each animal remained in this position was recorded.

### Microbiology

All the thia- and oxadiazolylureas (compounds **19**–**51** and **55**–**87**, respectively) were screened for antibacterial activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), and for antimycotic activity against *Candida albicans*, *C. krusei*, *C. parapsilosis*, and *T. glabrata*, according to the minimal inhibition concentration (*MIC*) method and the gradual redoubled dilution technique as described previously [27, 28].

Because of their low solubility in water, compounds were dissolved in dimethylsulfoxide and then appropriately diluted with the broth (Muller-Hinton and Sabouraud agar for bacteria and mycetes, respectively) by using variable concentrations ranging from 3.12–200 μg/ml.

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