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FIVE-MEMBERED HETEROCYCLIC AMINES AS POTENTIAL ANTI-RHEUMATOID ARTHRITIS AGENTS.

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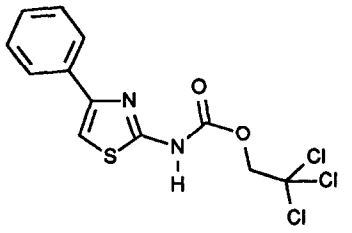
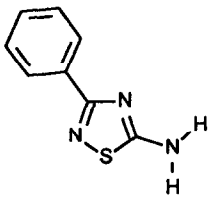
Abstract: 5-Amino-3-substituted-1,2,4-thiadiazoles have been found to reduce the inflammatory and arthritic symptoms in the adjuvant arthritis model of rheumatoid arthritis.

There are many reports in the literature on the antiarthritic or antiinflammatory activities of thiazoles, probably as a result of their immunostimulatory and in some cases platelet aggregatory modes of action.[1] We have found that when Lotifazole[2] **1** (4-phenyl-2-(2′,2′,2′-trichloroethoxy-carboxamido)-thiazole), a representative example of this class, is evaluated in terms of its effect on adjuvant arthritis in rats (AA-model) no reduction of the inflammatory response could be measured. Clearly this well established *in vivo* model[3] has failed to demonstrate the advantageous profile of activity that **1** has shown in alternative models.[2]



1 Lotifazole



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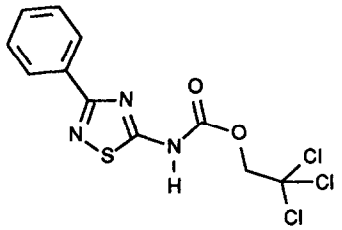
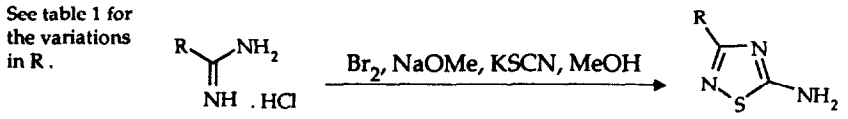


3

Our original investigations were performed with the intention of blocking metabolic oxidative cleavage at the 5-position on thiazole compounds[4], and we reasoned that placing a nitrogen heteroatom in this position might achieve this objective, probably make the corresponding 1,2,4-thiadiazole compounds less toxic and hopefully show a superior efficacy when compared with thiazole derivatives. The 5-amino-1,2,4-thiadiazoles described here were synthesised by the method of Goerdeler *et al.*[5] (Scheme 1). The first example 5-amino-3-phenyl-1,2,4-thiadiazole **2**, showed a significant improvement in joint mobility and a reduced swelling in the B- and C-lesions on the adjuvant arthritis test (Table 1), thereby demonstrating efficacy in this chronic model of inflammation. A 5-(2′,2′,2′-trichloroethoxy-carboxamido)-derivative **3** was also made by reacting **2** in THF with 2,2,2-trichloroethyl chloroformate and triethylamine as base. Interestingly this latter compound was not as active as **2**. The small series of amino-1,2,4-thiadiazoles described in this paper was produced from appropriate amidine hydrochlorides which were made either by the classical Pinner method[6] or by the method published by Weintraub *et al.*[7]

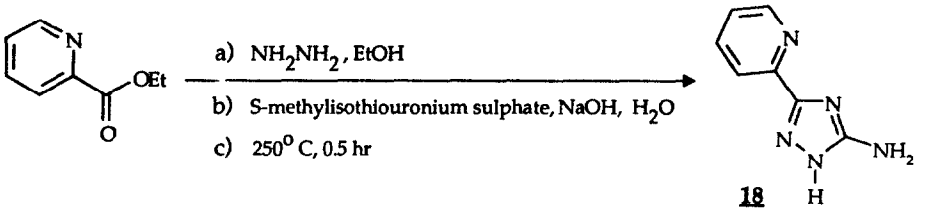
Scheme 1



The compounds were compared with cyclosporin-A as a standard, and compounds showing reductions in the C-lesion of greater than 50% were regarded as active (Table 1). Compound **4**, with no 3-substituent, appeared to be very potent on the AA-model, this was probably a manifestation of the toxicity of the compound, and some animals perished. This was the only 1,2,4-thiadiazole that was toxic in the entire series and may be a reflection of the vacant 3-position being exposed to possible metabolism leading to toxic species. Electron-withdrawing or electron-donating phenyl substituted examples (compounds **5** and **6** and others not included in the table) showed a diminution of activity when compared with **2**. A 3-methyl derivative **7** was active, although with more lipophilic alkyl members of the series (compounds **8** and **9**) activity was reduced, however **10**, a "hybrid" analogue of **2** and **7**, still retained significant activity. An initial perception, deduced from logP values (determined using the method of Garst *et al.*[8]), and solubilities measured at 25°C, in aqueous tyrode solution (where pH is buffered to 7.5), was that compounds which were relatively hydrophilic were the most potent. When subsequent compounds were examined however, (*e.g.* compound **10** with logP = 2.05), there were many exceptions to this first impression and clearly other factors are important. That some aqueous solubility is necessary however can be seen when comparing the solubilities of for example **5**, **6** and **10**, which when measured (in tyrode solution) were 80, <10 and 1274 μg. ml^{-1} respectively. The heterocyclic examples also demonstrate that factors other than hydrophilicity are important, *i.e.* the thiophene compound **11** had only marginal activity (logP = 1.77, solubility = 350 μg ml^{-1}) and the pyrimidine and pyrazine examples **15** and **16** were found to be inactive (logP = -0.08, 0.52, and solubilities = >2000, 170 μg. ml^{-1} respectively). An interesting comparison can be made with the 2- and 4-pyridyl examples, **12** and **14** (logP = 1.72, 1.58 and solubilities = 132, 1834 μg.ml^{-1} respectively), which are both active and the 3-pyridyl compound **13** (logP = 1.05, solubility = 172 μg. ml^{-1}), which is inactive. Clearly electronic influences are important here.

When 3-amino-1,2,4-triazoles were compared with the 5-amino-1,2,4-thiadiazoles compound **17** [9] was also active and it is noteworthy that this unsubstituted example apparently showed no toxicity (*c.f.* compound **4**). The 5-(2-pyridyl)-analogue **18**, which was originally synthesised by Lipinski[10], (Scheme 2), was also approximatly equipotent with compound **14**.

Scheme 2



18

The synthesis used by Plenkiewicz *et al.* to make 5-amino-3-(2-pyridyl)-1,2,4-oxadiazole **19** is very elegant[11,12], unfortunately this compound was not very active on the AA-model (*c.f.* **14** and **18**). However this synthesis could be exploited to make a 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-

derivative **20** in 49% yield, (Scheme 3) this is a moiety of considerable interest[13], and **20** was one of the most active members of the series. Compound **20**, was reported in the literature by Unangst *et al.* [14], and made by a different procedure in much lower yield (16%). They found it to be a moderately potent dual inhibitor of the cyclo-oxygenase and 5-lipoxygenase enzyme pathways (IC_{50} of 2.5 and 3.0 μM respectively). We have confirmed this result using the techniques described by Osborne *et al.*[15], but we have also found that none of the other members of this series inhibited these enzymes (except compound **21**). This *in vitro* activity appears to be due to the 3,5-di-*tert*-butyl-4-hydroxyphenyl-moiety and was responsible for the dual inhibitory mechanism of action of many other similar compounds made by these authors[14,16]. Even the acetylated derivative of **20** compound **21** showed significant AA-activity, a reverse of the normal trend in the series where any derivatisation of the amino-group causes lowering of activity. This is further evidence that the 3,5-di-*tert*-butyl-4-hydroxyphenyl-moiety has altered the *in vitro* activity and contributed to the *in vivo* AA activity of **20** and **21**.

Scheme 3

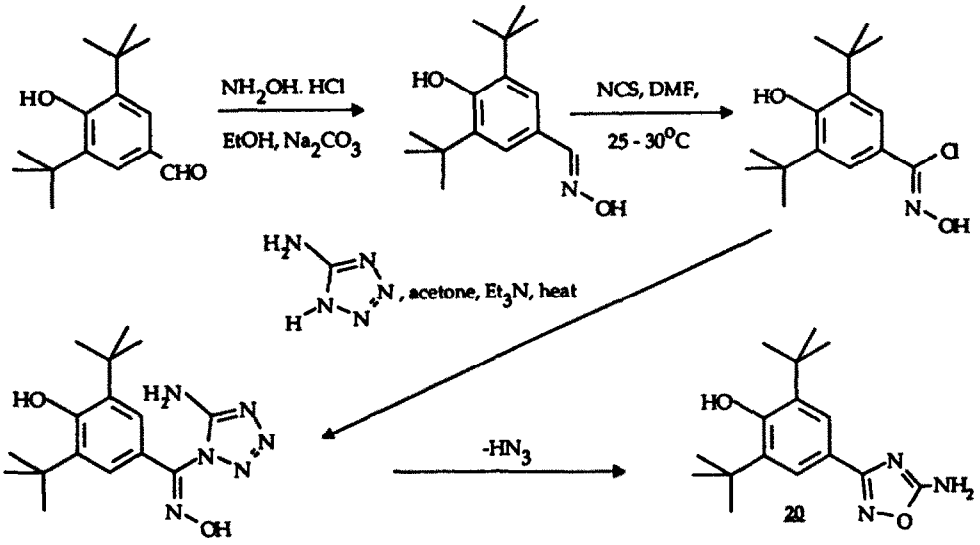
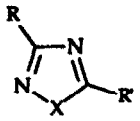
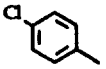
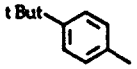
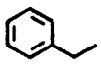
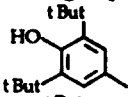
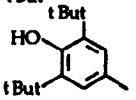


Table 1 **Effect of compounds on adjuvant arthritis in rats**[a]



no.	logP	R	R'	X	% reduction in paw volume				mp/°C	lit. ref.
					A	B	C	J.M.		
2	1.81		NH_2	S	-22	-51[c]	-84[c]	-13		5
3			TCECA	S	-50[b]	-45	-68[c]	-42	166-168	

Continued on the following page.

no.	logP	R	R'	X	% reduction in paw volume				mp/°C	lit. ref.
					A	B	C	J.M.		
4	-0.66	H	NH_2	S	-45[c]	-59	-106[c]	-69		5
5	2.83		NH_2	S	-20	-50	-46	-18	182-183	
6	1.78		NH_2	S	27	-2	6	18	194-196	
7	0.00	Methyl	NH_2	S	-38[c]	-50[b]	-66[c]	-84		5
8	1.12	^{n}Propyl	NH_2	S	-13	-40	-54	-47		17
9	1.78	^{t}Butyl	NH_2	S	-16	-30	-45	-28	158-162	
10	2.05		NH_2	S	-28[b]	-42[c]	-77[c]	-36		5
11	1.77		NH_2	S	-6	-25	-43[c]	-7	196-199	
12	1.72		NH_2	S	11	-20	-74[b]	-33		18
13	1.58		NH_2	S	-15	-22	49	-24		19
14	1.05		NH_2	S	-8	-44[c]	-65[c]	-70		19
15	-0.08		NH_2	S	-28[c]	-31[c]	-5	-30	249-251	
16	0.52		NH_2	S	-10	17	24	6	288-290	
17		H	NH_2	NH	-27[b]	-46[b]	-57[b]	-26		9
18			NH_2	NH	-3	-32[b]	-60[b]	-21		10
19			NH_2	O	24	22	66	8		12
20	3.72		NH_2	O	-47[b]	-56[b]	-103[b]	-89		14
21			AcAm	O	80	-66[b]	-79[b]	-75	236-237	
22		cyclosporin A			-51	-68	-77	-100		

Notes on the adjuvant arthritis data: [a] At oral doses of 33 mg. Kg^{-1}, except cyclosporin A which was dosed at 10 mg. Kg^{-1}. A = right primary lesion, the volume change in the injected paw measured from day 0 to day 8; B = right primary lesion, the volume change in the injected paw measured from day 9 to day 18; C = left secondary lesion, the volume change in the uninjected paw, measured from day 9 to day 18; all reductions in paw volume were denoted as negative values. J.M. = joint mobility, improvements in J.M. were denoted as negative values. All measurements were made using Sprague Dawley rats.
TCECA = 2',2',2'-trichloroethoxycarboxamido, AcAm = acetylamido. [b] $P < 0.001$, [c] $P < 0.01$.

Discussion

The metabolic fate of a ^{14}C labelled analogue of compound **14** was studied in F344 rats, which were dosed intravenously. Over 80% of the dose was recovered in urine during the 96 hours of this study period. Approximately 40% of this was parent compound with at least 4 unidentified polar metabolites, which were not conjugates of glucuronic acid or sulphate. However, the compound was metabolically stable when it was subjected to *in vitro* studies using a liver homogenate preparation. This result when compared with the *in vivo* information suggests that there may be involvement of extrahepatic drug metabolising enzyme systems. Since the prevention of oxidative cleavage at the C-5 position on this thiadiazole has not been demonstrated, the original concept has not been proven although, of course, the data provided shows that amino-thiadiazoles are active on an AA-model when amino-thiazoles are not.

The biological mechanism of action of the triazoles and the thiadiazoles is uncertain. As was discussed earlier, none of them was found to inhibit either cyclooxygenase or lipoxygenase. This is also so for the oxadiazoles with 3-substituents, the exceptions are compounds **20** and **21** which possess the 3,5-di-*tert*-butyl 4-hydroxyphenyl-moiety. All of the compounds described did not inhibit the T-lymphocyte response to conconcavalin A, or delay the process of graft versus host rejection, apparently ruling out any immunoregulatory mode of action, (*c.f.* Lotifazole[2]).

Reduction in paw volumes and increased spleen weights were seen with male Lewis rats (200-225 gm.) in compounds **7** and **14**, in a lipoidal amine arthritis assay as used by Benslay and Bendele[20]. The lipoidal amine (LA) (7.5 mg. per animal) used by these authors can induce a form of arthritis similar to that seen in the classical adjuvant disease, it is however a severe test of a compound since (LA) is dosed 9 days before an animal is treated with the material (50 $mg.Kg^{-1}$). Treatment is stopped after 13 days. Although the decrease in paw volumes is therefore very encouraging, when considering the severity of the assay, the increase in splenomegaly observed with these compounds (though not significant) may indicate that they are showing some weak NSAID activity, albeit by a mechanism that differs from most of the classical NSAIDS.

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