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SYNTHESIS AND ANTI-ARTHRITIC ACTIVITY OF A SERIES OF 1-ARYL-3-DIMETHYLAMINO-1,4-DIHYDROISOQUINOLINES

J. Bermudez, I. Hughes, E. H. Karran, F. R. Mangan, R. E. Markwell*, S. A. Smith, M. J. Thomson and P. A. Wyman

SmithKline Beecham Pharmaceuticals, Discovery Research,

Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, U.K.

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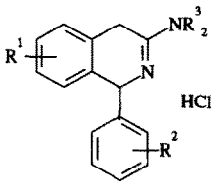
Abstract. 1-Aryl-3-dimethylamino-1,4-dihydroisoquinolines (**1**) were synthesised from 1-aryl-1,4-dihydroisoquinol-3-ones (**2**) by heating in dimethylcarbamyl chloride. Compounds bearing electron withdrawing substituents on the 1-aryl ring were active in inhibiting polyarthritis in the rat adjuvant arthritis model, when administered orally. Several compounds were also potent inhibitors of inflammatory cell accumulation. The most potent compound of the series, overall, was the 3'-chloro analogue, **10**.

In rheumatoid arthritis, the pathological destruction of joint bone and cartilage is associated with an intense infiltration of inflammatory cells, especially monocytes and neutrophils.[1] These cells are able to release degradative enzymes, oxygen radicals, and inflammatory cytokines such as Interleukin-1 and Tumour Necrosis Factor-α that may, by a variety of mechanisms, mediate connective tissue degradation.[2-4] There is great interest in identifying novel anti-arthritic agents that can inhibit the destruction of joint connective tissue, and one therapeutic approach receiving considerable attention at present is the pharmacological regulation of immune and inflammatory cell trafficking.[5]

As amidines[6] and guanidines[7] had previously been reported to possess anti-inflammatory and disease-modifying[8] activities, we screened a variety of novel amidines and identified a series of 1-aryl-3-dimethylamino-1,4-dihydroisoquinolines (**1**), which are active, after oral administration, in inhibiting both the primary and secondary phases of arthritis in the rat adjuvant arthritis model and, in addition, inhibit inflammatory cell accumulation.



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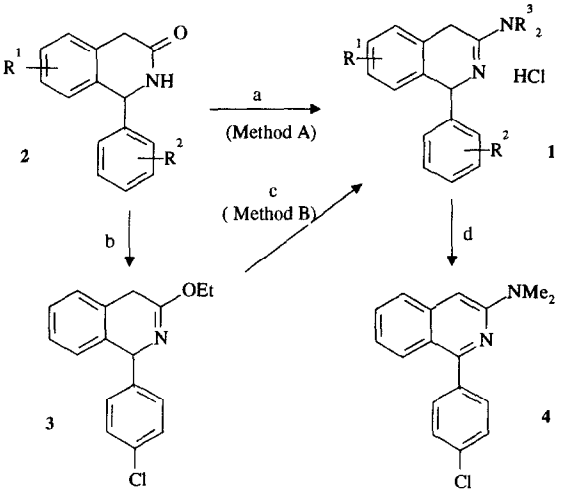
Compounds (Table 1) were synthesized as shown in Scheme 1. The key lactam intermediates **2**, were prepared following literature procedures,[9] and heated in neat dimethylcarbamyl chloride[10] to afford amidine hydrochlorides (**5, 9-14, 16, 17**), with N,N-dimethyl substitution (Method A). Yields were generally in the range 30-50%. Compounds (**6-8**), with alternative amidine N-substitution, were prepared by reaction of **2** with triethyloxonium tetrafluoroborate[11] to afford the unstable lactim-ether **3** followed by reaction with an appropriate amine[12] (Method B). The 4'-amino anologue **15** was prepared by hydrogenation of the 4'-nitro-compound **13** over palladium-carbon. The phenol **18** was obtained from the methyl ether **17** by heating in 48% hydrobromic acid, and the isoquinoline **4** was prepared from the dihydroisoquinoline hydrochloride **5** by oxidation with activated manganese dioxide.[13]

Scheme 1 Synthetic Route to Dihydroisoquinolines



a) Me_2NCOCl / 150 - 180 °C b) $Et_3O^+BF_4^-$ then Na_2CO_3 - H_2O
c) $R^3{}_2NH$ - EtOH d) MnO_2 - CH_2Cl_2

Compounds were tested by oral administration in the rat adjuvant arthritis model[14] (Table 1). The initial lead compound, **5**, (N,N-dimethyl amidine substituent) was highly inhibitory at a dose of 12.5 mg/kg but activity was lost when the amidine N-substituents were varied (compounds **6-8**). Potency was reduced if the 4'-chloro moiety was replaced by other electron-withdrawing substituents (**9-13**); compound **10**, with a 3'-chloro-substituent, being about half as potent. Activity was lost, however, when the 4'-chloro-substituent was replaced by hydrogen (**14**) or an amino (**15**) group. From testing more than twenty compounds with 1-phenyl substituents selected from all four quadrants of the Craig Diagram[15] (full data not shown) it appears that substituents with lipophilic (π) and electronic (σ) values[16] in the ranges (-0.6 to 1.4) and (0.2 to 0.8) respectively, are required for activity in the adjuvant arthritis model. The effect of introducing substituents into the dihydroisoquinoline ring was shown by comparing compounds **16** (7-Cl) and **17** (6-OMe), where only the

former compound was active. Compound **18,** with a 6-hydroxy substituent, was active but only when dosed intra-peritoneally. The isoquinoline analogue of **5** (compound **4**) was only weakly active, highlighting the importance of the amidine moiety for high potency.

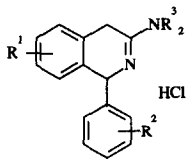


Table 1 Rat Adjuvant Arthritis Data

cmpd[a]	R^1	R^2	R^3	dose mg/kg, p.o.	max. inhibitory effect (%) injected paw depth[b]	max. inhibitory effect (%) arthritis score[c]
5	H	4-Cl	Me	12.5 5	80*** 30*	44** 18
6	H	4-Cl	H	25	0	-3
7	H	4-Cl	morpholino	25	7	5
8	H	4-Cl	H,CH_2Ph	25	10	-7
9	H	2-Cl	Me	25 12.5	30* 12	36*** 10
10	H	3-Cl	Me	25 10	84** 32*	53** 29**
11	H	2-F	Me	25	13	37*
12	H	2,4-di Cl	Me	12.5	44*	15
13	H	4-NO_2	Me	12.5	33**	30*
14	H	H	Me	25	-6	13
15	H	4-NH_2	Me	25	6	9
16	7-Cl	4-Cl	Me	25 6	86*** 36*	54*** 34**
17	6-MeO	4-Cl	Me	25	0	11
18 (HBr)	6-OH	4-Cl	Me	50 25 (i.p.)	5 46***	16 44***
4[d] (Free base)	H	4-Cl	Me	25	-1	29***

[a] Compounds **5**, **9-14**, and **16-17** prepared by Method A. Compounds **6-8** prepared by Method B by reacting lactim ether **3** with NH_3, morpholine and $PhCH_2NH_2$ respectively.
[b,c] * $p< 0.05$, ** $p<0.01$, *** $p<0.001$ [b][Student's 't' test] [c][Mann Whitney 'U'].
[d] isoquinoline structure

Several of the compounds were also examined in models of cell accumulation in the rat: the 72 h carrageenin-induced pleurisy model;[17] the implanted polyvinyl sponge model;[18] and the reversed passive Arthus reaction[19] (Table 2). In the carrageenin-induced pleurisy model, the monocyte is the predominant cell type in the exudate fluid, whereas the neutrophil is the major cell type present in the polyvinyl sponge model. In the Arthus reaction, the complement cascade is activated at the site of immune complex deposition, resulting in

neutrophil accumulation and oedema. Compounds **9**, **10** and **11** were active in all three models; compound **10** (3'-Cl substituent) was especially potent, with significant activity at 3-6 mg/kg (p.o.) but, by contrast, the phenol **18**, even given i.p. (*cf* Table 1), was considerably less active.

Table 2 Pharmacology Data for 1-Aryl-3-dimethylamino-1,4-dihydroisoquinolines

cmpd	pleural exudate model [a,b]		polyvinyl sponge model[a,c]		Arthus reaction[a,d]	
	dose mg/kg p.o.	% inhibition	dose mg/kg p.o.	% inhibition	dose mg/kg p.o.	% inhibition
5	25 12.5 6	28** 21*** 19*	25 12.5	30* 15	NT[e]	
9	25 12.5 6	48** 28* 27**	25	19*	50 25	34** 19
10	12.5 6 3	41* 41* 36**	10 5	35* 8	50 25 12.5 6.25	42** 29* 30* 31*
11	12	33*	25 10	24*** 12	50 25 12.5	55*** 44** 28*
18	25 (i.p.)	21	NT[e]		50 (i.p.)	18

[a] * p< 0.05, ** p<0.01, *** p<0.001 [Mann Whitney 'U'], [b] For method see ref.17 [c] For method see ref.18. [d] For method see ref.19. [e] NT = Not tested

The mode of action of the dihydroisoquinolines is presently unknown. It is well known that compounds that interfere with arachidonic acid metabolism, such as the non-steroidal anti-inflammatory drug indomethacin, are able to inhibit the adjuvant arthritis model.[20] To investigate this potential mechanism of action, several members of the series (including **9** and **18**) were tested for their ability to inhibit prostaglandin synthetase obtained from bovine seminal vesicles,[21] and against 5-lipoxygenase extracted from RBL1 cells[22] and in both tests the amidines were more than fifty fold less potent than the standard inhibitors, indomethacin, BW 755C and nordihydroguaiaretic acid (NDGA) (data not shown). Prostaglandin synthetase inhibitors and dual cyclooxygenase/lipoxygenase pathway inhibitors have previously been reported to *potentiate* cellular accumulation in the rat pleurisy model,[17] and it is therefore very unlikely that these compounds are inhibiting cell accumulation by directly affecting prostanoid production. As there are reports on the anti-complement activity of amidines, *e.g.* FUT-175,[6a] the mode of action of the compounds in the reversed passive Arthus model was investigated by determining their effects on complement activation in an *in vitro* haemolysis assay.[23] Several members of the series (including **10, 12** and **16,**) were shown to be only weakly active ($IC_{50} > 10^{-5}$ M), implying that the compounds do not act by directly inhibiting the complement system.

When dosed repeatedly in the adjuvant arthritis model, many of the more lipophilic compounds induced behavioural changes consistent with an effect on the central nervous system. However, these side-effects were

substantially abrogated in the more hydrophilic phenol **18**, and this compound lacked acute toxicity at doses up to 90 mg/kg when dosed intraperitoneally into mice.

Dihydroisoquinolines such as **10** are potent inhibitors of both neutrophil and monocyte cell accumulation, and possess profound anti-arthritic activity in the rat adjuvant arthritis model. A more extended study of the mechanisms by which these compounds act may provide insights into the processes involved in cell migration. In addition, these compounds may be useful pharmacological tools for elucidating the role that inflammatory cell accumulation plays in the pathogenesis of immune and inflammatory diseases.

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(13) All compounds gave satisfactory ^{1}H NMR, and E.I. mass spectra data, and elemental analysis (± 0.4 %). The ^{1}H NMR data for compound **9** , which is representative of the series, are as follows: [80 MHz; $CDCl_3$-$(CD_3)_2SO$] δ 3.23 (s, 3 H), 3.4 (s, 3 H) (NMe_2), 4.36 (m, 2 H)(1-H_2), 6.32 (m, 1 H)(4-H), 6.6-7.6 (m, 8 H)(aromatics) and 10.2 (m, 1 H)(NH^+). ^{1}H NMR data for the isoquinoline **4** : [80 MHz; $CDCl_3$] δ 3.25 (s, 6 H) (NMe_2), 6.7 (s, 1 H)(1-H), and 7.05-8.0 (m, 8 H)(aromatics).

(14) The test was carried out as described by Newbould, B. B. *Brit. J. pharmacol.* **1963**, *21*, 127-136. TUCK Wistar male rats (200-230 g bodyweight) received subplantar injections into the right hind paw of 0.1 mL of a 4 mg/mL suspension of *Mycobacterium tuberculosis* in liquid paraffin. Compounds were administered orally, or intraperitoneally, for 14 days starting on the day of adjuvant injection. The development of the arthritis was monitored by measurement of the injected paw depth, and also by means of an arthritic score, reflecting the number of joints affected and the severity of the disease. The maximum percentage inhibitory effects observed for the test compounds, compared to the arthritic controls, were determined during the period of the polyarthritis (days 10 - 17).

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(17) The method of Vinegar, R.; Truax, J. F.; Selph, J. L.; Voelker, F. A. *Fed. Proc.* **1982**, *41*, 2588-2595 was followed. A 2% solution (0.2 mL) of γ-carrageenin (Viscarin 402) was injected intrapleurally in anaesthetised rats (175-200 g). Test compounds were administered orally in methylcellulose, or intraperitoneally in 0.9% saline, 1 h before carrageenin, and at 24 and 48 h after carrageenin injection. After 72 h, a solution (4 mL) of EDTA (5 g) and phenol red (0.32 g) in 0.9 % saline (1 L) was injected into the pleural cavity after killing the animals. The exudate fluid and saline wash was removed and the DNA content measured. The results are expressed as the percent inhibition of the treated group compared to the vehicle-dosed control group.

(18) This model of neutrophil accumulation was a modification of the method of Ford-Hutchinson, A. W.; Walker, J. R.; Smith, M. J. H. *J. Pharm. Meth.* **1978**, *1*, 3-7. Sterile polyvinyl sponges (Declon 49, 10±1 mg each) were implanted under anaesthesia into groups of 10 male Wistar rats (150 - 180 g) with each rat receiving one sponge subcutaneously on either side of a ventral mid line incision. Six hours later, the sponges were removed and their cellular content measured. The test compounds were administered, orally in methylcellulose, 0.5 h prior to sponge implantation and the results are expressed as the percent inhibition of the treated group compared to the vehicle-dosed control group.

(19) The assay was that of Chang, Y.; Otterness, I. *Eur. J. Pharmacol.* **1981**, *69*, 155-164. Groups of male Wistar rats were injected through the tail vein with 0.5 mL of a 4 mg/mL solution of ovalbumin in 0.9 % saline. The animals were then injected subplantar with 0.1 mL of a solution of saline into the left hind foot, and with 0.1 mL of a solution of rabbit anti-ovalbumin antiserum into the right hind foot. The animals were then immediately dosed with the test compounds orally in methylcellulose, or intraperitoneally in 0.9% saline. The swelling due to the Arthus reaction was assessed by calculating the difference between the volumes of the hind feet, 3 h after subplantar injection. The results are expressed as the percent inhibition of the treated group compared to the vehicle-dosed control group.

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