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SYNTHESIS AND IMMUNOSUPPRESSANT ACTIVITY OF PYRAZOLE CARBOXAMIDES

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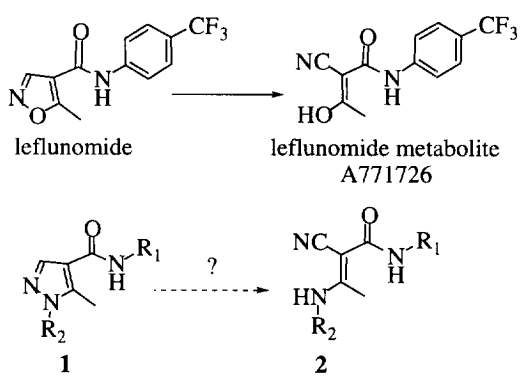
Abstract: A series of novel pyrazole carboxamides is disclosed that demonstrate strong immunosuppressant activity in rodent and human mixed leukocyte response (MLR) assays ($IC_{50} < 1$ μM). The synthesis, biological activity, mode of action, and pharmacokinetic properties of this new lead series are discussed.

Immunosuppressant agents such as cyclosporin A and FK506 that act via inhibition of IL-2 production have been employed for the treatment of autoimmune diseases and for prevention of organ transplant rejection for more than two decades.[1] Despite their widespread use, these treatments have shown undesirable side effects[2,3] and hence the search for a new generation of immunosuppressants has continued. Smaller molecules such as leflunomide and brequinar are representative of newer immunosuppressants, which act via inhibition of dihydroorotate dehydrogenase (DHODH). Unfortunately, dose-limiting toxicity (brequinar)[4–6] or slow clearance (leflunomide)[7,8] has resulted in unusual dose regimens or drug monitoring protocols for both compounds. These clinical difficulties have led to continued efforts toward the discovery of potent, novel immunosuppressant drugs with fewer adverse side effects for transplantation/autoimmune therapy.

The isoxazole immunosuppressant leflunomide is quickly metabolized to its ring opened isomer A771726 believed to be the active therapeutic agent (Figure 1).[9] We surmised that a similar process might occur in pyrazoles where the nitrogen at the N-1 position was mortgaged to an appropriate substituent. The pyrazole 4-carboxamides bearing aromatic groups at N-1 have shown immunosuppressant activity equivalent to leflunomide and brequinar. In this report we describe the synthesis of these pyrazole analogs and discuss their SAR and mode of action in comparison with leflunomide.[10]

Figure 1.



Chemistry

Our initial interest focused on the N-1 substituent of the heterocyclic pyrazole core, which was prepared via the diketene route in Scheme 1.[11–13]

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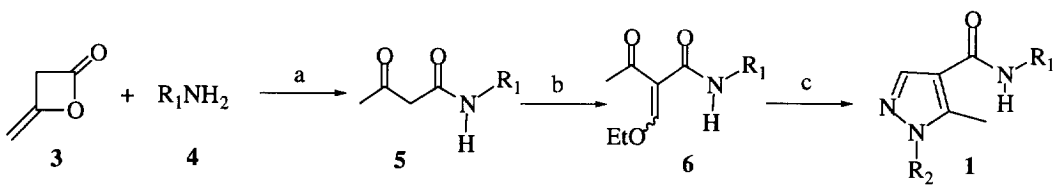
Scheme 1

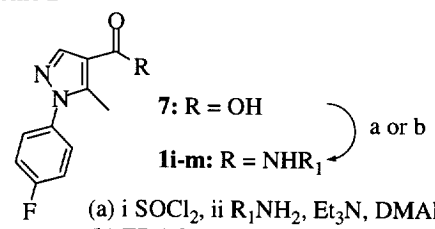


(a) toluene, 60 °C; (b) $CH(OEt)_3$, Ac_2O, reflux; (c) R_2NHNH_2, EtOH, reflux

Anilines and amines **4** were condensed with diketene (**3**) in good yields, and the resulting acetoacetamides **5** were treated with triethyl orthoformate to deliver enol ethers **6**. Condensation with a variety of hydrazines provided the desired 1-substituted-5-methyl pyrazole-4-carboxamide analogs **1** in good yield with high regioselectivity (usually >95:5).[10]

Regiochemistry of the N-1 phenyl derivatives was determined via NOE experiments which showed an interaction of the N-1 substituent with the C-5 methyl group in the major regioisomer. Altenative routes included activation of **7** (which was similarly prepared from diketene) via conventional methods[14,15] followed by treatment with amines **4** provided amides **1i–m** (Scheme 2).

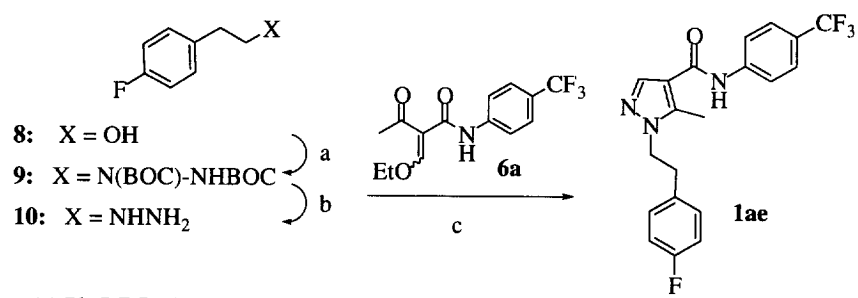
Scheme 2



(a) i $SOCl_2$, ii R_1NH_2, Et_3N, DMAP, DCM, rt; (b) EDAC, R_1NH_2, Et_3N, DCM, 0° C → rt.

Deviation from 1-phenyl pyrazole derivatives required a number of alicyclic hydrazines. Those hydrazines not commercially available were prepared using the Mitsunobu protocol as depicted in Scheme 3 for 4-fluorophenethyl analog **1ae**.[16,17]

Scheme 3



(a) Ph_3P/DBAD, THF, rt; (b) HCl(g) Et_2O; (c) NaOH, EtOH

Synthetic hydrazines such as **10** were condensed with enol ethers such as **6a** to afford the desired pyrazoles.[18]

Biological Assays

The heterocyclic amides were assayed in the rat and human mixed leukocyte response assay (MLR, Table 1).[19] MLR assays are in vitro models of allogeneic T cell activation wherein peripheral blood mononuclear leukocytes (PBLs) from a single individual are exposed to a pool of mitomycin C-treated PBLs from randomly selected individuals. After 4-5 days, the incorporation of ^{3}H-thymidine into cellular DNA is measured. Active immunosuppressants inhibit proliferation thereby reducing the amount of thymidine incorporation when compared to untreated controls. The MLR assay is a fundamental benchmark for immunosuppressant activity, and data allow comparisons between species.

At the pyrazole N-1 position a phenyl ring appears to be optimal. Many substituents are tolerated in the *ortho* and *meta* positions of the phenyl ring yet the *para* position is best suited with electron withdrawing groups. Insertion of a tether between the pyrazole and the phenyl ring is also tolerated, (**1ac–1ae**) yet all of these N-1 alkyl pyrazoles require a phenyl terminus to maintain efficacy. The 4-fluorophenyl group was among the most active in MLR and the fluorine in the para position precluded metabolism of the aromatic ring. Consequently this substituent was preferred among the N-1-phenyl groups,.

Alteration of the amide portion of the compounds was extensive. Most substituted carboxanilides as well as amides derived from bulky hydrophobic groups showed some activity. However, insertion of a tether between the heterocyclic amide and the phenyl terminus diminishes activity. Overall, the preferred derivatives were the 4-trifluoromethyl secondary anilides such as **1a**.[20]

Mode of Action Studies

As the SAR proceeded, we investigated whether these pyrazoles were operating via the same mode of action as isoxazoles. Although a variety of in vitro effects have been observed for leflunomide,[21, 22] compelling studies describe inhibition of pyrimidine biosynthesis via suppression of dihydro-orotate dehydrogenase (DHODH).[23–30] These inhibitory effects are reversed by addition of exogenous uridine. Using a similar uridine-reversal study pyrazole **1a** was unaffected by exogenous uridine in a Jurkat T cell proliferation assay. As shown in Figure 2, under conditions where added uridine reversed the inhibitory effect of leflunomide, pyrazole **1a** maintained a uridine-independent antiproliferative efficacy.

Figure 2. Reversal of inhibition with added uridine (1mM) in Jurkat cells.

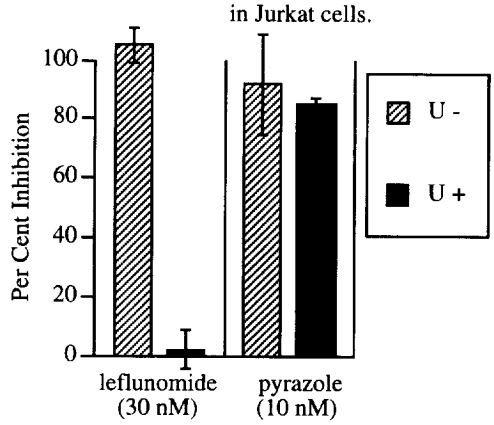


Table 1. Mixed Leukocyte Response (MLR) data for test compounds [a]

Compound	R_1	R_2	RMLR	HMLR
1a	4-CF_3 Ph	4-F Ph	0.27	0.85
1b	4-F Ph	4-F Ph	0.61	2.40
1c	4-Me Ph	4-F Ph	0.20	0.54
1d	4- CF_3O Ph	4-F Ph	2.60	3.40
1e	3-F Ph	4-F Ph	0.80	3.00
1f	3-MeO Ph	4-F Ph	3.60	3.70
1g	3-Me Ph	4-F Ph	0.89	5.70
1h	3- CF_3 Ph	4-F Ph	1.10	1.70
1i	2-F Ph	4-F Ph	5.10	4.00
1j	2-Me Ph	4-F Ph	0.11	1.10
1k	4-F benzyl	4-F Ph	0.33	na [b]
1l	4-F phenethyl	4-F Ph	0.30	na
1m	3-heptyl	4-F Ph	0.11	0.58
1n	4- CF_3 Ph	H	na	na
1o	4- CF_3 Ph	Ph	0.65	2.30
1p	4- CF_3 Ph	4-MeO Ph	1.00	0.34
1q	4- CF_3 Ph	4-Me Ph	1.00	0.41
1r	4- CF_3 Ph	4-Cl Ph	7.40	0.41
1s	4- CF_3 Ph	4-NO_2 Ph	2.30	5.70
1t	4- CF_3 Ph	3-F Ph	0.22	3.10
1u	4- CF_3 Ph	3-Me Ph	0.89	2.50
1v	4- CF_3 Ph	3-MeO Ph	2.50	2.50
1w	4- CF_3 Ph	2-F Ph	0.11	1.10
1x	4- CF_3 Ph	2-Me Ph	0.15	1.50
1y	4- CF_3 Ph	2- CF_3 Ph	0.26	2.60
1z	4- CF_3 Ph	2,3-di Cl Ph	0.24	1.60
1aa	4- CF_3 Ph	3,5-di Me Ph	0.41	2.20
1ab	4- CF_3 Ph	3-Cl-4-F Ph	0.21	1.30
1ac	4- CF_3 Ph	benzyl	2.70	3.70
1ad	4- CF_3 Ph	4-F benzyl	2.40	3.60
1ae	4- CF_3 Ph	4-F phenethyl	1.70	5.40
2a	4- CF_3 Ph	4-F Ph	na	na
2o	4- CF_3 Ph	Ph	na	na
2s	4- CF_3 Ph	4-NO_2 Ph	na	na
Leflunomide			1.00	5.40
Brequinar			1.60	3.90

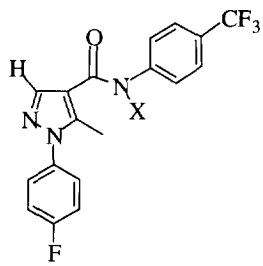
[a] Rat (RMLR) or Human (HMLR) mixed leukocyte response IC_{50} (μM). Values represent an average of at least six replicates
[b] na = Not active <20% inhibition @ 10 μM

Subsequently we examined the inclination of these compounds to form the proposed metabolites **2**. Despite rigorous attempts,[10] comparable ring opening could not be chemically induced. Independently prepared[31,32] pyrazole 'metabolites' **2a, 2n,** and **2s** were inactive in MLR assays (Table 1) and they could not be detected in vivo during pharmacokinetic evaluation of the parent compounds. Although these pyrazoles were modeled after isoxazoles inclined to form ring-opened metabolites, these compounds are not prone to heterocyclic ring opening as a prerequisite for biological activity. The pyrazoles are thus a distinct class of immunosuppressants which do not operate via inhibition of DHODH.[33]

Bioavailability

While SAR led to a variety of compounds with in vitro activity comparable to reference immunosuppressants, many of the analogs in this series showed poor bioavailability, which may be due to insufficient aqueous solubility. Consequently, solubilizing groups were installed in tolerant positions of compound **1a**. This strategy proved successful with compound **1af** where the addition of a carboxylic acid increased the solubility 3800-fold with a concomitant improvement the PK profile (Figure 3) while preserving immunosuppressive activity.[20]

Figure 3. Solubilities and pharmacokinetic parameters for **1a** and **1af**



Compound	1a	1af
X	H	CH_2CO_2H
Aqueous Solubility (μg/mL @ pH 7.3)	0.4	1533
T 1/2 (h)	13.7	6.0
C Max (μg/mL)	0.46	4.45
AUC (μg•h/mL)	7.0	31.8
RMLR (IC_{50})	0.27	0.31
HMLR (IC_{50})	0.85	3.20

Conclusions

The pyrazole carboxamides represent a novel class of immunosuppressive agents with unique SAR and mode of action which are equipotent with newer reference immunosuppressants. SAR to date indicates that N-phenyl amides at C-4 are optimal, and the N-1 position can be varied provided a phenyl group remains, although a direct phenyl linkage is preferred. The lack of uridine-dependence suggests that inhibition of DHODH is not a likely mode of action for the heterocycles. We have investigated tertiary amide analogs where installation of solubilizing functions resulted in an enhanced pharmacokinetic profile. Bioavailable analogs of this series will be further studied in in vivo models of immunosuppression.

References and Notes

1. Wahrenberger, A. *Crit. Care Nurs. Q.* **1995**, *17*, 27.
2. Treleaven, J. G.; Barrett, A. J. *Br. J. Hosp. Med.* **1990**, *43*, 256.
3. Rees, A. J.; Lockwood, C. M. *Clinical Aspects of Immunology*; Blackwell Scientific: Oxford, 1982; p 507.
4. Schwartsmann, G.; van der Vijgh, W. J.; van Hennik, M. B.; Klein, I.; Vermorken, J. B.; Dodion, P.; ten Bokkel Huinink, W. W.; Joggi, G.; Gall, H.; Crespeigne, N.; et al. *Eur. J. Cancer Clin. Oncol* . **1989**, *25*, 1675.
5. Noe, D. A.; Rowinsky, E. K.; Shen, H. S.; Clarke, B. V.; Grochow, L. B.; McGuire, W. B.; Hantel, A.; Adams, D. B.; Abeloff, M. D.; Ettinger, D. S. *Cancer Res.* **1990**, *50*, 4595.

6. de Forni, M.; Chabot, G. G.; Armand, J. P.; Fontana, X.; Recondo, G.; Domenge, C.; Carde, P.; Barbu, M.; Gouyette, A. *Eur. J. Cancer* **1993**, *29a*, 983.
7. Furst, D. E. *Baillieres Clin. Rheumatol* . **1995**, *9*, 711.
8. Mladenovic, V.; Domljan, Z.; Rozman, B.; Jajic, I.; Mihajlovic, D.; Dordevic, J.; Popovic, M.; Dimitrijevic, M.; Zivkovic, M.; Campion, G.; Musikic, P.; Low-Friedrich, I.; Oed, C.; Seifert, H.; Strand, V. *Arthritis & Rheumatism* **1995**, *38*, 1595.
9. Bartlett, R. R.; Dimitrijevic, M.; Mattar, T.; Zielinski, T.; Germann, T.; Rude, E.; Thoenes, G. H.; Kuchle, C.; Schorlemmer, H. U.; Bremer, E.; Finnegan, A.; Schleyerbach, R. *Agents Actions* **1991**, *32*, 10.
10. A preliminary account of this work has been presented: Wang, A. X.; Xie, Q.; Lane, B.; Mollison, K. W.; Hsieh, G. C.; Marsh, K.; Sheets, M. P.; Luly, J. R.; Coghlan, M. J. *Abstracts of papers*, 212th National Meeting of the American Chemical Society ACS, Orlando, FL, 1996; Abstract MEDI 187.
11. Doleschall, G.; Seres, P. *J. Chem. Soc., Perkin Trans.* **1988**, *1*, 1.
12. Menozzi, G.; Mosti, L.; Schenone, P. *J. Heterocycl. Chem.* **1987**, *24*, 1669.
13. Jackson, A. H.; Shannon, P. V. R.; Wilkins, D. J. *J. Chem. Soc., Chem. Commun.* **1987**, 653.
14. Beckwith, A. L. In *The Sythesis of Amides*; Zabicky, J. Ed.; J. Wiley & Sons: New York, 1968; pp 75–119.
15. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C.; Loffet, A. *Proc. 14th Eur. Pept. Symp.* **1976**, 79.
16. Demers, J. P.; Klaubert, D. H. *Tetrahedron Lett.* **1987**, *28*, 4933.
17. Guillerm, D.; Linstrumelle, G. *Tetrahedron Lett.* **1985**, *26*, 3811.
18. Alternate routes to the 1-alkyl analogs involving N-alkylation of unsubstituted pyrazoles delivered separable mixtures of 3- and 5-methyl regioisomers.
19. Kawai, M.; Lane, B. C.; Hsieh, G. C.; Mollison, K. W.; Carter, G. W.; Luly, J. R. *FEBS Lett.* **1993**, *316*, 107.
20. In addition to the increased Cmax and AUC, pyrazole **1af** demonstrated a 71% bioavailability in this rat study.
21. Cherwinski, H. M.; McCarley, D.; Schatzman, R.; Devens, B.; Ransom, J. T. *J. Pharmacol. Exp. Ther* . **1995**, *272*, 460.
22. Bartlett, R. R.; Dimitrijevic, M.; Mattar, T.; Zielinski, T.; Germann, T.; Rude, E.; Thoenes, G. H.; Kuchle, C. C.; Schorlemmer, H. U.; Bremer, E. *Agents Actions* **1991**, *32*, 10.
23. Cao, W. W.; Kao, P. N.; Chao, A. C.; Gardner, P.; Ng, J.; Morris, R. E. *J. Heart Lung Transplant.* **1995**, *14*, 1016.
24. Cherwinski, H. M.; Byars, N.; Ballaron, S. J.; Nakano, G. M.; Young, J. M.; Ransom, J. T. *Inflamm. Res.* **1995**, *44*, 317.
25. Cherwinski, H. M.; Cohn, R. G.; Cheung, P.; Webster, D. J.; Xu, Y. Z.; Caulfield, J. P.; Young, J. M.; Nakano, G.; Ransom, J. T. *J. Pharmacol. Exp. Ther* . **1995**, *275*, 1043.
26. Chong, A. S.; Rezai, K.; Gebel, H. M.; Finnegan, A.; Foster, P.; Xu, X.; Williams, J. W. *Transplantation* **1996**, *61*, 140.
27. Davis, J. P.; Cain, G. A.; Pitts, W. J.; Magolda, R. L.; Copeland, R. A. *Biochemistry* **1996**, *35*, 1270.
28. Greene, S.; Watanabe, K.; Braatz-Trulson, J.; Lou, L. *Biochem. Pharmacol* . **1995**, *50*, 861.
29. Knecht, W.; Bergjohann, U.; Gonski, S.; Kirschbaum, B.; Loffler, M. *Eur. J. Biochem* . **1996**, *240*, 292.
30. Xu, X.; Williams, J. W.; Gong, H.; Finnegan, A.; Chong, A. S. *Biochem. Pharmacol* . **1996**, *52*, 527.
31. Shvo, Y.; Shanan-Atidi, H. *J. Am. Chem. Soc.* **1969**, *91*, 6689.
32. Shvo, Y.; Shanan-Atidi, H. *J. Am. Chem. Soc.* **1969**, *91*, 6683.
33. Compound **1a** was also studied in several other well defined assays with established MOAs. Studies related to IL-2 synthesis by T cells, IL-2-induced T cell proliferation, B cell proliferation, leukotriene biosynthesis, and modulation of tyrosine kinase activity were undertaken yet compounds such as **1a** show little activity in any of these experiments. Determination of the MOA of these pyrazoles remains an area of active interest.