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SYNTHETIC INHIBITORS OF INTERLEUKIN-6 I: 2,3,7,8-TETRAHYDRO-4-ARYL-1H-CYCLOPENT [e] IMIDAZO [1,2-a]- PYRIDIN-5(6H)-ONE AND RELATED COMPOUNDS

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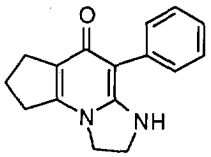
Abstract : A versatile synthetic route to the title class of compounds and the development of an orally absorbed analogue of the lead structure are described. The minimum structural requirements needed in the compounds related to **1** for the inhibition of interleukin-6 were identified.

Lymphocytes (consisting of the T and B cells) and other cells of the immune system produce certain proteins which are soluble mediators of immune and inflammatory response. These proteins which are often inter-dependent in eliciting their biological effects are collectively called cytokines.[1] Interleukins are a family of cytokines that govern leukocyte function in response to any challenge to the immune system.[2] Over-expression of interleukins has been implicated in a variety of inflammatory diseases; their modulation, particularly via small molecules, is currently of therapeutic interest.[3]

Interleukin-6 (IL-6) is an ubiquitous and multi-functional cytokine involved in the early steps of T-cell activation, B-cell differentiation, in the regulation of the acute phase response (which is characterized by the release of a set of serum proteins in response to injury) and in hematopoiesis (the maturation of blood cells from bone marrow). Human IL-6 (previously called IFN β-2, BCDF, BSF-II) is a 28kD protein which is synthesized as a 212 amino acid precursor that is processed into a 184 amino acid secreted product.[4] Consistently elevated levels of IL-6 are found in the synovial fluid associated with rheumatoid arthritis[5] and in multiple myeloma tumors.[6] Inhibitors of IL-6 synthesis / release and IL-6 receptor antagonists would have potential applications in the treatment of the above diseases. However, no small molecules which are *specific* inhibitors of IL-6 production or its effects have been reported to date. In this and in the accompanying paper, we describe preliminary studies on two different types of small molecules which inhibit the production of IL-6.

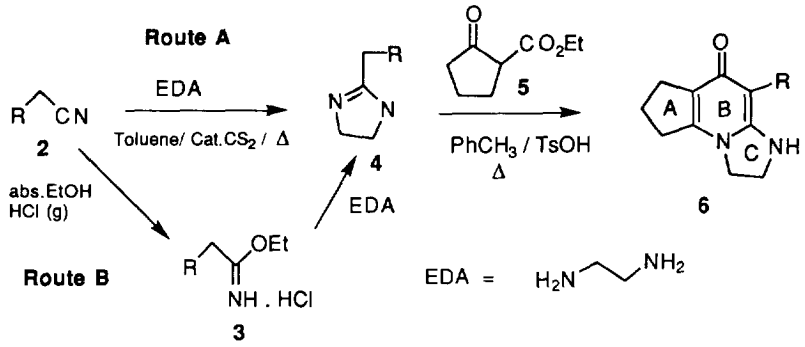


1. Sch-24471

Sch-24471 (**1**) was identified as an inhibitor of IL-6 through the screening of our compound file. Details of a synthetic program aimed at targets related to **1** and the ensuing the structure-activity relationship are presented here.

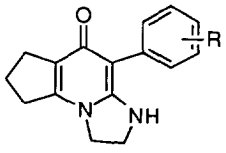
Chemistry: As shown in Scheme 1, the reaction of imidazolines **4** with cyclic β-keto esters **5** formed the tricyclic pyridinones of the generic structure **6**. The imidazolines in turn were obtained from the corresponding nitrile **2** (route A) or the imidate ester **3** (route B) both of which are readily available.[7]

Scheme 1

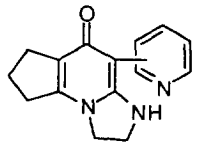


Compounds in which the cyclopentane (ring-A) was replaced with six and seven membered rings or deleted altogether were similarly prepared and had no activity; likewise, replacement of the imidazoline (ring-C) with the homologous pyrimidine resulted in loss of activity. Thus, at the outset, we decided to keep the core of structure **1** intact and study the effect of the following changes on anti-IL-6 activity: (i) Substituents on the phenyl ring. (ii) A spacer between the tricyclic core and the phenyl ring. (iii) Replacing the phenyl ring with other hetero-aromatic rings as well as with H, COOR etc. (iv) Replacing the NH in ring-C with N-R, S and O.[8] Structures of the compounds synthesized for this study are depicted in Scheme 2.[9]

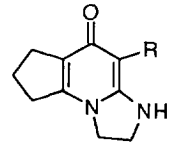
Scheme 2 : Structures of Representative Analogues of **1**



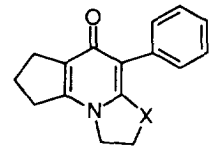
7. R = 2-OMe
8. R = 2-Cl
9. R = 2-OH
10. R = 2-CF_3
11. R = 2-NO_2
12. R = 2-NH_2
13. R = 2-$NHCOCH_3$
14. R = 3-COOEt
15. R = 3-COOH



16. 2-Pyridyl
17. 3-Pyridyl



18. R = SPh
19. R = SMe
20. R = CO_2Et
21. R = H



22. X = N-Me
23. X = N-Bn
24. X = S
25. X = O

Compounds **11**, **12**, **14** and **18** were prepared by route-B; All others by route-A.

Synthesis of compound **25** is outlined in ref. 8.

Biology : All compounds were screened at 10μM for the inhibition of lipopolysaccharide(LPS)-induced IL-6 production in murine myelomonocytic leukemia (WEHI-265.1) cells. The level of IL-6 was assessed by ELISA.[10] Compounds which exhibited atleast 70% inhibition at 10μM were then tested several (≥3) times to confirm activity and obtain an IC_{50}; less active compounds were tested ≤3 times. These results are summarized in Table 1(SD = Standard Deviation). Selected compounds were then progressed to an in vivo assay in which they were tested at 25 mg/kg of bodyweight (mpk), given intraperitoneally (i.p) or orally (p.o) for the inhibition of LPS induced IL-6 in the serum of C3H mice. In this assay, compounds were given 1 hour prior to an i.p injection of LPS (50μg); sera were collected 4 hours after the LPS and IL-6 content was determined by ELISA.

Table 1 : In Vitro IL-6 Inhibitory Activity of Compounds Listed in Scheme 2

Compd. #	% Inhibition @ 10 μM (±SD)	Compd. #	% Inhibiton @ 10 μM (±SD)	Compd. #	% Inhibition @ 10 μM (±SD)
1	74 (±10)	**13**	74 (±14)	**20**	58 (±25)
7	82 (±6)	**14**	83 (±14)	**21**	38
8	90 (±6)	**15**	0!	**22**	27
9	55	**16**	55	**23**	43
10	90 (±11)	**17**	0!	**24**	52 (±14)
11	73 (±14)	**18**	63	**25**	20
12	82 (±10)	**19**	77 (±21)	-	-

As can be discerned from the above table, 4-aryl substituted tricyclic pyridinone has been identified as the active pharmacophore which is required for the inhibition of IL-6 production. The presence of the phenyl ring is essential for activity (**1** vs. **20, 21**). Substitution on the phenyl ring is generally well-tolerated (**7-14**), exceptions being OH (**9**) and CO_2H (**15**). Ortho substitution appears to be better than meta- and para-substitution (these analogs of compounds **7-15** were made and tested but are not included here due to space limitations) with only one exception (3-CO_2Et, compound **14**).[11] This may be a steric rather than electronic effect as can be seen from the in-vitro activities of 2-Cl, 2-OMe, 2-CF_3, 2-NO_2 and 2-NH_2 . In searching for a metabolically more stable replacement for NO_2 or NH_2, we found that NHCOMe (**13**) is equally good. Other changes such as introducing a sulfur atom between the tricylic core and the phenyl ring (**18**), replacing the phenyl ring with a pyridine ring (**16,17**) as well as alkylating the imidazoline NH (**22,23**) or replacing it with sulfur / oxygen (**24/25**) all resulted in loss of activity. In vitro IC_{50} s were determined only for our best compounds : 0.2 μM (**1**), 4.0 μM (**8**), 6μM (**11**), 2.0 μM (**12**), 5.0μM (**13**) and 0.6μM (**14**).

Excellent in vitro activity did not always translate into comparable in vivo results. Compounds **10,14** and **19**, for example, were completely inactive in vivo. However, our initial lead compound **1** showed 77% (i.p) and 39% (p.o) inhibition of serum IL-6 at 25 mpk giving an ED_{50} of 0.35mpk (i.p) and 32 mpk (p.o). Compounds **7** and **8** had ED_{50} s of 0.3 and 0.5 mpk respectively when administered intrapertoneally, but had poor oral absorption. Compound **11** had 78% inhibition of serum IL-6 at 25 mpk (p.o) with an ED_{50} of 8 mpk(p.o). The

anilino derivative **12** was our best orally absorbed compound, exhibiting a 72% inhibition of serum IL-6 at 25 mpk with an ED_{50} of 4 mpk (p.o). This represents an eight fold increase over our initial lead in terms of oral absorption. It is also worth noting that this class of compounds had no significant in vitro activity against other cytokines (TNF-α, IL-1, IL-5 and γ-interferon) when tested under appropriate conditions[12], *indicating that they are not general inhibitors of cell function.* The preliminary study discussed herein would serve as the basis on which more potent compounds could be designed for extensive in vivo studies and mechanistic investigations.

Acknowledgement : We thank Dr. John Piwinski (SPRI) for discussions.

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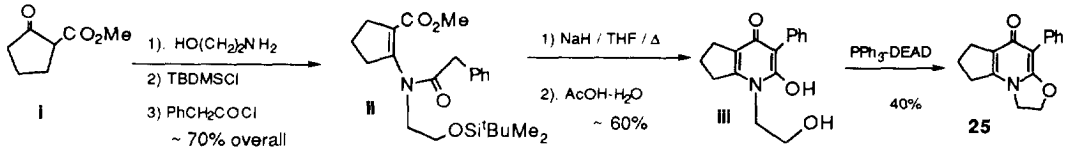
8. The synthesis of the oxygen analogue **25** using the general route failed. Its synthesis is depicted below :



9. All compounds reported here were fully characterized by spectral and analytical data. Melting points (°C) : **7** : 184-190; **8** : 214-216; **9** : 175-177; **10** : 223-226; **11** : 254-257; **12** : 120-123; **13** : 248; **14** : 199; **15** : 176; **16** : 168-170; **17** : 233-236; **18** : 199-201; **19** : 185-190; **20** : 192-194; **21** : 202-204; **22** : 174-176; **23** : 92-94; **24** : 178-180; **25** : 194-197
10. For details of the Enzyme-Linked ImmunoSorbent Assay (ELISA), see: Barton, B. E.; Jakway, J. P.; Smith, S. R.; Siegel, M. I. *Immunopharm. Immunotox.* **1991**, *13*, 251.
11. Synthesis of the ortho-ester in this series was problematic, but the meta and para esters were made by route-B. While the para ester was only moderately active, the meta ester **14** was more active than other meta substituted compounds; it is included here as the only "non-ortho" substituted compound to show interesting activity.
12. Barton, B. E. Unpublished data.

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