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Synthesis and Bioactivities of Novel Piperidylpyrimidine Derivatives: Inhibitors of Tumor Necrosis Factor-Alpha Production

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Abstract—New piperidylpyrimidine derivatives, including quinazolines, were prepared, and their abilities to inhibit TNF-α production evaluated. Some compounds showed potent inhibitory activity in mouse macrophages stimulated with LPS. The synthesis and structure–activity relationships of these compounds are described.

Tumor necrosis factor-α (TNF-α) is a key pro-inflammatory cytokine that is produced mainly by activated monocyte/macrophages in response to immunostimulants. Increasing evidence indicates that TNF-α is a major mediator of inflammatory and immune responses and that it has pleiotropic biological effects.[1] Elevated TNF-α levels also have been implicated in the pathogenesis of such diseases as septic shock,[2,3] cachexia,[4,5] rheumatoid arthritis (RA),[6,7] inflammatory bowel disease (IBD)[8] and AIDS.[9–11] Moreover, TNF-α is a strong inducer of such other pro-inflammatory cytokines as IL-1, IL-6 and IL-8.[7] Agents which inhibit TNF-α production therefore may have therapeutic potential for the direct or indirect treatment of these diseases. In clinical trials, monoclonal antibodies to TNF-α have been effective in the treatment of RA and Crohn's disease.[12–15] During screening for compounds that inhibit TNF-α production, benzoyl-substituted piperidylquinazolinone (**3**)[16] was found to have such activity. Using this as the lead compound, we obtained some modified compounds. We found that certain piperidylpyrimidines are potent inhibitors of TNF-α production in LPS-stimulated mouse macrophages. The synthesis and structure–activity relationships in this series of compounds are described.

Chemistry and Biology

The compounds in Tables 1–4 were synthesized as outlined in Schemes 1–4. Quinazoline derivatives modified at the C(2)-position and piperidine nitrogen were prepared as shown in Scheme 1. Benzoylation of 4-cyanopiperidine **1**[17] and subsequent selective *ortho* acylation[18] with aniline in the presence of boron trichloride and aluminum trichloride gave 2-aminophenyl ketone **2**. The compound **2** was cyclized with potassium cyanate and cyanamide, yielding the respective 2-oxo (**3**) and 2-amino (**4**) quinazolines. Non-aqueous diazotization-replacement of 2-aminoquinazoline **4** by the *tert*-butyl nitrite (TBN)-$SbCl_3$ system[19] gave 2-chloroquinazoline **5** which was converted to **6** by hydrogenolysis. Hydrolysis of **4**, followed by alkylation or acylation in the usual manner gave a series of substituted amino or amide compounds **8a–j**.

2,4-diaminoquinazoline derivatives **11a–c** were prepared from 2,4-dichloroquinazoline **9** by stepwise reactions with the requisite amines (Scheme 2).[20,21]

Pyrimidine derivatives modified at the C(6)-position were prepared as shown in Scheme 3. Condensation of the requisite ketones with the acid chloride of **12**[22] followed by cyclization with guanidine gave **14a** and **14b**. In the case of 4-methoxyphenylacetone, a 1:1 mixture of the 1,3-diketone isomers **13c** and **13d** was produced. Each of these chromatographically separable isomers could be processed to the desired pyrimidines, **14c** and **14d**, respectively (Scheme 4). Hydrolysis of **14c**, followed by acylation with the requisite acid chloride and triethylamine or the requisite acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSC) gave the series of amide compounds **16a–x**. The test compounds were purified by standard procedures like column chromatography and crystallization. Structures were confirmed with NMR and MS spectroscopy.

Compounds were evaluated for their abilities to inhibit TNF-α production in mouse macrophages stimulated

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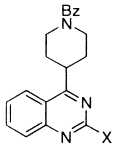
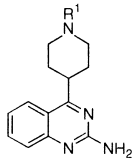
Table 1. SAR of 2-substitution



Compound	X	TNF-α inhibition IC_{50} (μM)
3	OH	20
4	NH_2	1.4
5	Cl	1.4
6	H	1.4

Table 2. SAR of piperidine substitution



Compound	R^1	TNF-α inhibition IC_{50} (μM)
7	H	51
8a	CH_3	>100
8b	Bn	16
8c	$Ph(CH_2)_2$	>100
8d	Cyclohexyl	53
8e	Ac	3.6
8f	$CH_3(CH_2)_3CO$	3.0
8g	Cyclohexanoyl	2.5
8h	$PhCH_2CO$	2.0
8i	$Ph(CH_2)_2CO$	6.3
8j	3,4-(OCH_2O)PhCO	4.5
11a		>100
11b		>100
11c		>100

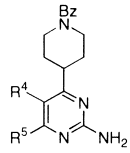
with LPS as described elsewhere.[23] Test results are given as the IC_{50} values (μM) of a single determination. Pentoxifylline[24–27] (Sigma Co.) was the positive control used to give an IC_{50} of 23 μM.

Results and Discussion

Compounds in which the substitution at the C(2)-position of the quinazoline ring varied are shown in Table 1. There was a marked increase in activity in this series as compared with **3**, the initial lead compound. The structural form of **3**, preferably the keto tautomeric form[28,29] depicted in Scheme 1, suggests that the electron-rich N(1)-nitrogen of quinazoline acts as a hydrogen-bond acceptor and that this nitrogen is important for activity.

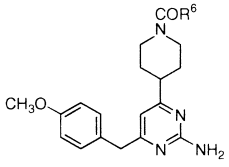
The various substituents on the piperidine nitrogen are shown in Table 2. In this series, the 2-substituent was fixed to the amino group taking into account both the activity and solubility of the compound. Acyl derivatives (**8e–j**) were tolerated despite the unsubstituted secondary amine (**7**), alkyl amino derivatives (**8a–d**) and amino derivatives (**11a–c**) directly substituted at the C(4)-position of quinazoline had diminished potency. The poor activities of these amino analogues suggest that this region must be nonbasic in order to be a potent TNF-α inhibitor.

Table 3. SAR of benzo fusion



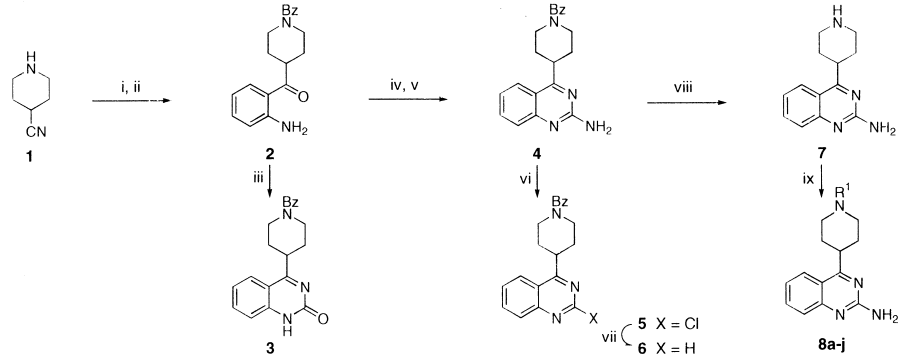
Compound	R^4	R^5	TNF-α inhibition IC_{50} (μM)
14a	H	CH_3	9.0
14b	H	Ph	5.1
14c	H	4-CH_3OPhCH_2	1.4
14d	4-CH_3OPh	CH_3	9.7

Table 4. SAR of piperidine substitution

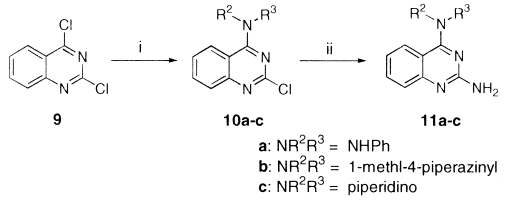


Compound	R^6	TNF-α inhibition IC_{50} (μM)
16a	CH_3	6.1
16b	CH_3CH_2	4.9
16c	$CH_3(CH_2)_2$	7.2
16d	$CH_3(CH_2)_4$	1.5
16e	$CH_3(CH_2)_6$	1.9
16f	*t*-Bu	5.6
16g	2-CH_3OPh	3.6
16h	3-CH_3OPh	1.2
16i	4-CH_3OPh	0.5
16j	2-NO_2Ph	1.7
16k	3-NO_2Ph	0.8
16l	4-NO_2Ph	0.7
16m	4-ClPh	0.6
16n	2,3-$(CH_3O)_2Ph$	0.9
16o	3,4-$(CH_3O)_2Ph$	0.9
16p	3,5-$(CH_3O)_2Ph$	1.0
16q	3,4-$(OCH_2O)Ph$	0.6
16r	3,4-Cl_2Ph	0.7
16s	$PhCH_2$	2.1
16t	4-CH_3OPhCH_2	1.7
16u	4-$ClPhCH_2$	2.4
16v	3,4-$(CH_3O)_2PhCH_2$	1.2
16w	3,4-$(OCH_2O)PhCH_2$	1.8
16x	3,4-Cl_2PhCH_2	2.1

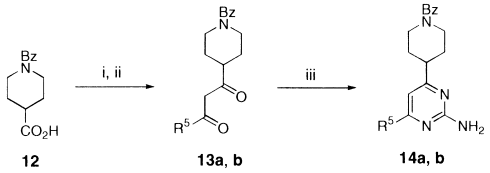
The results in Table 3 show the effect of substituted pyrimidines that dissociated the benzo moiety of quinazoline. Modification of the benzo moiety was tolerated, and of the analogues prepared, benzyl analogue **14c** had a potency equal to its benzo derivative **4**. Compound **14a** (R^4 = H, R^5 = Me) showed decreased potency, indicative of the importance of the lipophilic group for good potency. The lipophilic-substituted analogue **14d** (R^4 = 4-methoxyphenyl, R^5 = Me) in particular reduced activity relative to **14b** (R^4 = H, R^5 = Ph), indicative that a bulkier group at the C(5)-position of pyrimidine may distort the active conformation of the piperidine ring.



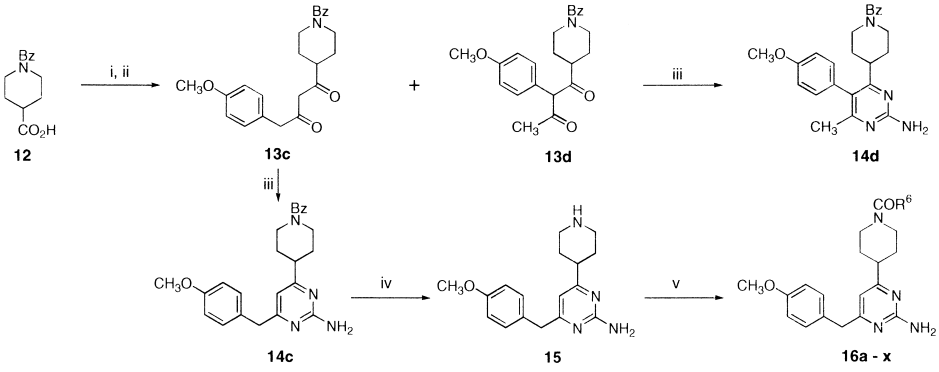
Scheme 1. Reagents and conditions: (i) BzCl, Et_3N, THF, 0 °C, 95%; (ii) aniline, BCl_3, $AlCl_3$, $(CHCl_2)_2$, 90 °C, 75%; (iii) KOCN, AcOH, 60 °C, 71%; (iv) HCl/Et_2O; (v) H_2NCN, 50 °C, 97%; (vi) *t*-BuONO, $SbCl_3$, $(CHCl_2)_2$, 60 °C, 32%; (vii) H_2, Pd/C, AcONa, MeOH, rt, 45%; (viii) 6N-NaOH, EtOH, reflux, 82%; (ix) R^1X, Et_3N, 0-80 °C, DMF, 20–41% (for **8a–c**); cyclohexanone, $NaB(CN)H_3$, HCl-MeOH, rt, 45% (for **8d**); Ac_2O, Et_3N, $CHCl_3$, 0 °C, 76% (for **8e**); RCOCl, Et_3N, 0 °C, $CHCl_3$, 45–69% (for **8f–j**).



Scheme 2. Reagents and conditions: (i) aniline, 1-methylpiperazine or piperidine, THF, rt, 75–95%; (ii) NH_3, EtOH, 90 °C, autoclave, 21–47%.



Scheme 3. Reagents and conditions: (i) $SOCl_2$, $CHCl_3$, reflux; (ii) R^5COCH_3 /LDA, THF, −70 °C, 38–54%; (iii) guanidine hydrochloride, K_2CO_3, pyridine, 110 °C, 74–77%.



Scheme 4. Reagents and conditions: (i) $SOCl_2$, $CHCl_3$, reflux; (ii) 4-methoxyphenylacetone/LDA, THF, −70 °C, 35% (for **13c**), 35% (for **13d**); (iii) guanidine hydrochloride, K_2CO_3, pyridine, 110 °C, 45% (for **14c**), 7% (for **14d**); (iv) 6N-NaOH, EtOH, reflux, 79%; (v) R^6COCl, Et_3N, CH_2Cl_2 or $CHCl_3$, 0 °C, 68–100% (for **16a–g**, **16i–m**, **16s**); R^6CO_2H, HOBt, WSC, $CHCl_3$, rt, 86–100% (for **16h**, **16n–r**, **16t–x**).

Substitutions at the piperidine nitrogen were further examined with the benzylpyrimidine derivative **14c** as the template. The various amide substituents are shown in Table 4. Alkanoyl derivatives (**16a–f**) were tolerated but did not produce more potent compounds. The mono- and di-substituted benzoyl analogues (**16g–r**) generally enhanced potency. Of these compounds, **16i**, **16k-o**, **16q** and **16r** had high potencies ($IC_{50} < 1$ μM). There was no marked difference in activity produced in terms of the electron-withdrawing or electron-donating nature of the benzoyl substitution (**16g–i** versus **16j–l**). The position of the substituent may, however, be important to the increase in activity in the order of *para*>*meta*>*ortho*. Phenylacetyl analogues (**16s–x**) showed activity that was weaker than that of the corresponding substituted benzoyl analogues.

In conclusion, a number of analogues of the piperidylquinazoline and piperidylpyrimidine derivatives were evaluated for their abilities to inhibit TNF-α production. Starting from weakly active lead **3**, we have identified potent inhibitors in both types of compounds. There is not marked difference in activity between the two structures, but the pyrimidine analogues are favorable from the view point of toxicity.[30] Therefore efforts to expand the structure–activity relationship are in progress using pyrimidines as substrates.

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