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# Design and synthesis of potent carboxylic acid DGAT1 inhibitors with high cell permeability

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ABSTRACT

A series of potent carboxylic acid DGAT1 inhibitors with high permeability were developed from a virtual screening hit. Strategies were employed to reduce Pgp substrate recognition and increase passive permeability, resulting in the discovery of a series showing good inhibition of cellular triglyceride synthesis. The mutagenic potential of prospective metabolites was evaluated in the Ames assay, and one aniline was shown to be devoid of mutagenicity.



For most of human history, mankind has struggled with food scarcity, and historically obesity has been viewed as a sign of wealth and prosperity. In the western world in the 21st century, food is abundant for most people, and obesity is now a leading preventable cause of death worldwide. At least 1.1 billion adults, and 10% of children are now overweight or obese.[1] Obesity can lead to decreased life expectancy due to increased risk of cardiovascular disease, Type 2 diabetes and certain cancers.

Diacylglycerol acyltransferase 1 (DGAT1) and DGAT2 catalyse the final and only committed step in triglyceride synthesis.[2] DGAT1 deficient mice are resistant to diet induced obesity, and display increased energy expenditure and reduced adiposity.[3] In contrast, DGAT2 deficient mice are profoundly lipopenic and die shortly after birth due to an impaired permeability function of the skin.[4] These findings have lead a number of companies to pursue selective DGAT1 inhibitors for the treatment of obesity and related metabolic disorders. For example, treatment of rodents with small molecule **1** has reproduced some characteristics of DGAT1 deficient mice (Fig. 1).[5] A number of DGAT1 inhibitors such as PF-04620110 (**2**) have entered clinical trials for obesity and metabolic disorders.[6]

Ligand-based virtual screening identified triazole **3**, a low molecular weight (MWt 382) inhibitor of the human DGAT1 enzyme, $IC_{50}$ 0.34 μM (a ligand efficiency of 0.33 based on the $IC_{50}$). Early profiling showed **3** to have selectivity over DGAT2 ($IC_{50}$ > 20 μM) and aqueous solubility of >1 mg/ml, in part due to a measured Log*D* of −2.1 at pH 7.4.[7] A medicinal chemistry program was initiated to build on the encouraging data and properties for **3**. Recent analysis of a large Caco-2 permeability dataset would suggest that given the molecular weight and polarity of **3**, it is unlikely to be highly permeable.[8] Initially we sought to explore the SAR with an aim to increase the potency of **3** by making modifications on the left hand side of the molecule via amide formation. The unusually low Log*D* of the starting hit, together with the potential need to seek more balanced physiochemical properties lead us to target analogues with increased lipophilicity/reduced polarity. Indeed, the Pfzier clinical candidate, PF-04620110, a carboxylic acid with measured Log*D* −0.15, has reportedly poor passive permeability.[9] One hazard of pursuing this strategy is that an increase in lipophilicity is frequently accompanied by increasing clearance, however this series of molecules exhibited high stability toward both mouse and human microsomes.

The synthesis of compounds **3**–**9** is outlined in Scheme 1. Construction of the triazole ring was carried out by reaction of *p*-nitrobenzoyl chloride with 4-methylthiosemicarbazide followed by cyclisation with sodium hydroxide. Functionalisation of the thiol with an α-bromoester, followed by reduction of the nitro group with tin afforded the aniline building block for amide formation. Finally, the acids were obtained by hydrolysis of the corresponding ester.

Many of these analogues (Table 1) demonstrated good inhibition of human DGAT1 enzyme. Hydrophobic substituents generally gave an enhancement of potency. In particular, extension of the

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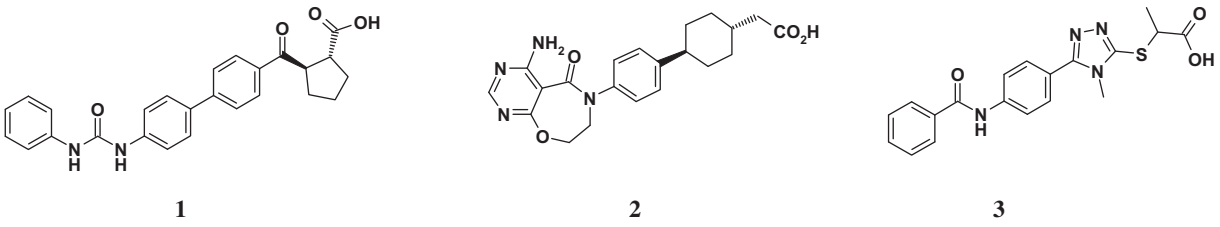
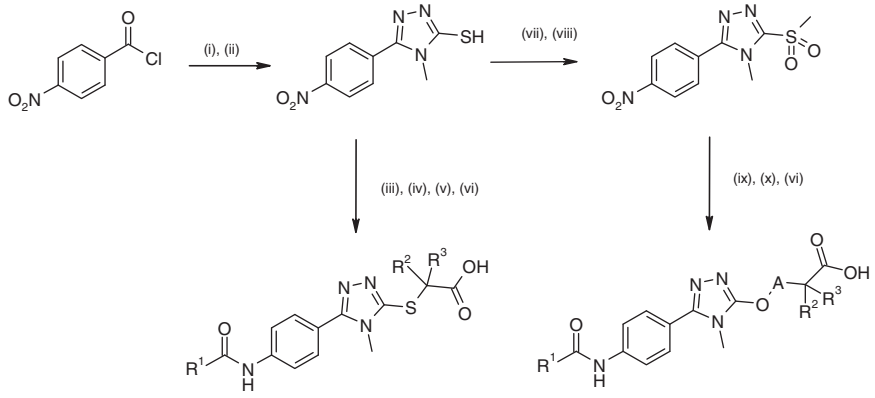


1 2 3

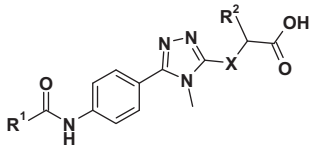
PF-04620110

**Figure 1.** Structures of some DGAT1 inhibitors.



**Scheme 1.** Reagents and conditions: (i) 4-Methylthiosemicarbazide, pyridine, DCM; (ii) 2 M sodium hydroxide, reflux, 1 h; (iii) α-bromoester, sodium methoxide, methanol; (iv) tin, hydrochloric acid; (v) acid chloride, THF, triethylamine; (vi) sodium hydroxide, methanol, room temperature ; (vii) MeI, sodium methoxide; (viii) m-CPBA, DCM, room temperature; (ix) Alcohol, NaH, THF, reflux, 90 min; (x) hydrogen, palladium on carbon, methanol.

**Table 1**
Effect of structural modifications on in vitro properties



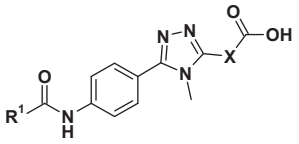
| Compd[a] | $R^1$ | $R^2$ | X | DGAT1 $IC_{50}$[b] (μM) | 3T3L1 $IC_{50}$[b] (μM) | ACD Log*D* pH 7.4 |
|---|---|---|---|---|---|---|
| **3** | Phenyl | Me | S | 0.35 | >20 | −0.53 |
| **4** | *p*-Tolyl | Me | S | 0.13 | >20 | −0.08 |
| **5** | *o*-Tolyl | Me | S | 1.98 | >20 | −0.08 |
| **6** | 2-Naphthyl | Me | S | 0.02 | >20 | 0.70 |
| **7** | 2-Benzothiophene | Me | S | 0.013 | >20 | 1.55 |
| **9** | 3,4-Dichlorophenyl | Me | S | 0.23 | >20 | 0.88 |
| **10** | 3,4-Dicholorbenzyl | Me | S | 0.038 | >20 | 0.52 |
| **11** | 3,4-Dichlorophenyl | Ph | S | 1.12 | >20 | 2.00 |
| **12** | 3,4-Dichlorophenyl | Et | S | 0.011 | >20 | 1.44 |
| **13** | 2-Naphthyl | Me | O | 0.144 | >10 | −0.29 |

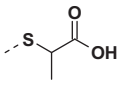
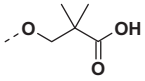
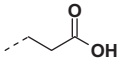
[a] All compounds were >95% pure by LC–MS and characterised by NMR and MS. All compounds are racemates.
[b] $N = 2$; for assay details see WO Patent 2008099221.

benzene to a fused biaryl (eg., **6** and **7**) gave at least an order of magnitude enhancement in potency. The 3,4-dichlorobenzamide, designed as a biaryl mimic, gave a slight increase in potency. Despite excellent inhibition of the DGAT1 enzyme, the compounds showed no significant inhibition of cellular triglyceride synthesis in mouse 3T3L1 adipocytes. Caco-2 permeability data for **9** (Table 2) showed it underwent significant efflux (efflux ratio >50).

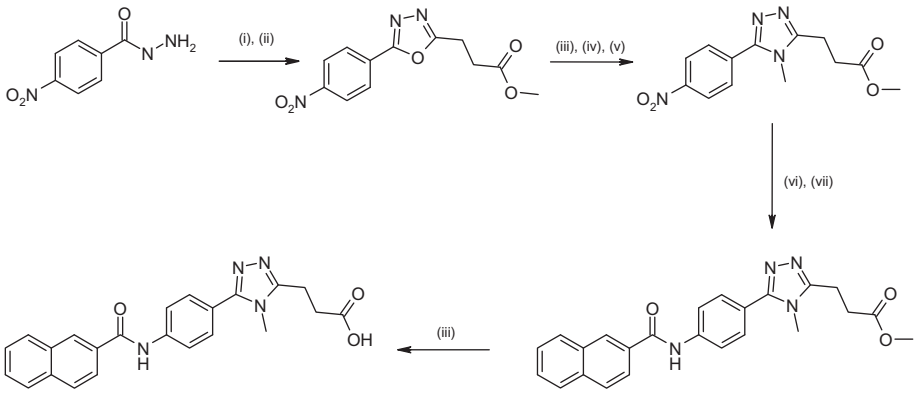
A number of strategies to prevent P-glycoprotein (Pgp) recognition of substrates (and hence reduce efflux) are described in the literature. Compounds with (N+O) ⩾8 and MWt >400 are likely to be Pgp substrates.[10a] Reducing heteroatom count could help mitigate Pgp recognition, and may also increase passive permeability, an approach to limiting the effect of Pgp.[10b] A number of structural modifications were made in an attempt to increase passive permeability, and reduce efflux. To reduce polarity (by increasing the $pK_a$ of the carboxylic acid)[11] an additional methylene spacer was introduced between the heteroatom and the acid. At the same time the sulphur was replaced with oxygen to remove a potential metabolic liability. The preparation of these analogues is outlined in Scheme 1. The thiol was converted to a leaving group via methylation and

**Table 2**
Effect of side chain modification on in vitro properties



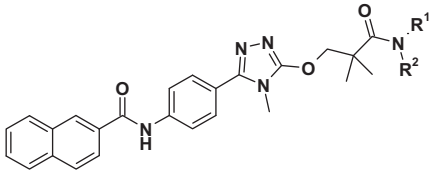
| Compd | $R^1$ | X | DGAT1 $IC_{50}$ (μM) | 3T3L1 $IC_{50}$ (μM) | Log*D* pH 7.4 | Caco-2 permeability | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | A–B | B–A | Efflux ratio |
| **9** | 3,4-Dichlorophenyl |  | 0.23 | >20 | 0.62 | <0.02[a] | 1.8[a] | >90 |
| **14** | 2-Naphthyl |  | 0.004 | 1.96 | 0.79 | 0.22[b] | 17.6[b] | 80 |
| **15** | 2-Naphthyl |  | 0.167 | 4.0 | 0.07 | 0.53[a] | 3.19[a] | 6 |

[a] Conducted at Cerep; permeability classification: A–B < $2.0 \times 10^{-6}$ cm/s = low; A–B $2.0 \times 10^{-6}$ cm/s to $20 \times 10^{-6}$ cm/s = medium and A–B > $20 \times 10^{-6}$ cm/s = high.
[b] Conducted at Absorption Systems; permeability classification: A–B < $1.0 \times 10^{-6}$ cm/s = low and A–B > $1.0 \times 10^{-6}$ cm/s = high.



**Scheme 2.** Reagents and conditions: (i) Methyl-4-chloro-4-oxobutanoate, DCM, triethylamine; (ii) phosphorous pentoxide, toluene, reflux 2 h; (iii) sodium hydroxide, THF, water; (iv) methylamine trifluoroacetate, 150 °C, 4 h; (v) methanol, concentrated sulphuric acid, reflux, 8 h; (vi) hydrogen, palladium on carbon, methanol; (vii) 2-naphthyl chloride, triethylamine, THF.

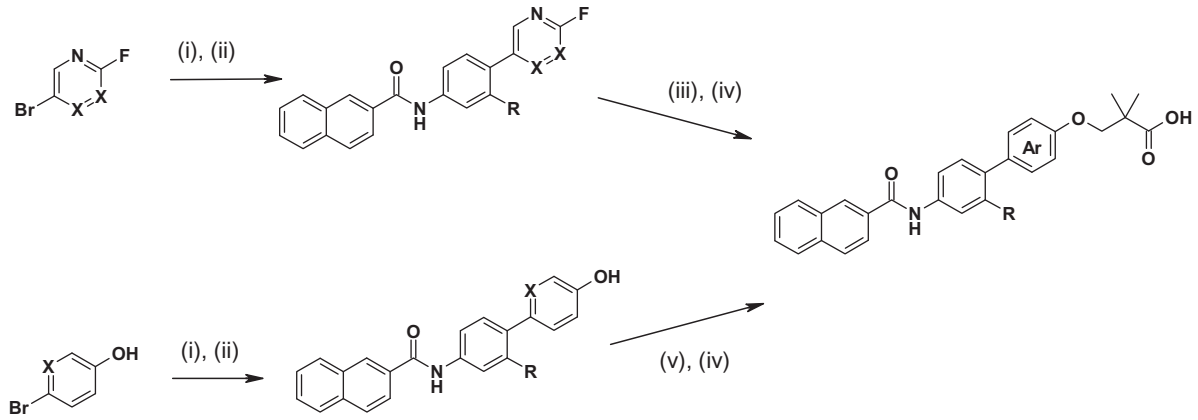
**Table 3**
In vitro evaluation of some triazole amides



| Compd | $R^1$ | $R^2$ | DGAT1 $IC_{50}$ (μM) | 3T3L1 $IC_{50}$ (μM) | ACD Log*D* pH 7.4 | HBD[b] | Caco-2 permeability[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | A–B | B–A | Efflux ratio |
| **16** | H | H | 0.028 | 6.1 | 3.9 | 3 | 0.10 | 47.9 | 479 |
| **17** | Me | Me | 0.029 | 0.44 | 4.4 | 1 | 21.9 | 56.4 | 2.6 |
| **18** | H | *i*Pr | 0.039 | 2.34 | 4.9 | 2 | 8.8 | 48.8 | 5.5 |
| **19** | H | Ph | 0.11 | 0.22 | 6.2 | 2 | 19.8 | 29.3 | 1.5 |
| **20** | H | $(CH_2)_2NMe_2$ | 0.053 | 5.89 | 3.6 | 2 | 0.21 | 8.11 | 38 |

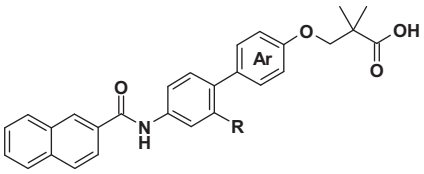
[a] Conducted at Absorption Systems; permeability classification: A–B < $1.0 \times 10^{-6}$ cm/s = low and A–B > $1.0 \times 10^{-6}$ cm/s = high; efflux ratio ⩾3 indicates significant efflux.
[b] HBD = Hydrogen bond donor count.

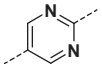
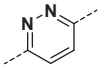
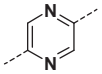
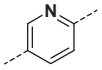
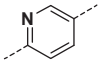
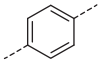
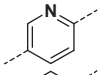
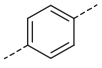
subsequent oxidation to the sulphone. The sulphone could then be displaced with an alcohol in the presence of base. A direct exchange of the sulphur atom for oxygen (compare **6** and **13**) resulted in a drop in potency. However, incorporation of an




**Scheme 3.** Reagents and conditions: (i) 4-Aminobenzene boronic acid, $Cs_2CO_3$, $PdCl_2$(dppf), dioxan, water 90 °C, 17 h; (ii) 2-naphthoyl chloride, triethylamine, THF; (iii) 3-hydroxy-2,2-dimethylpropionic acid methyl ester, sodium hydride; (iv) sodium hydroxide, THF, water; (v) 3-bromo-2,2-dimethylpropionic acid methyl ester, sodium hydride, DMF.

**Table 4**
Structure–activity relationships for some biaryl acids



| Compd | Ar | R | DGAT1 $IC_{50}$ (μM) | 3T3 $IC_{50}$ (μM) | ACD Log*D* pH 7.4 | Caco-2 permeability[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | A–B | B–A | Efflux ratio |
| **21** |  | Me | 0.115 | 0.433 | 1.7 | 33 | 62.1 | 1.8 |
| **22** |  | Me | 0.373 | 0.709 | 1.1 | 42.5 | 55.8 | 1.3 |
| **23** |  | Me | 0.117 | 2.16 | 1.9 | 19.3 | 38.8 | 2.0 |
| **24** |  | Me | 0.097 | 0.125 | 2.5 | — | — | — |
| **25** |  | Me | 2.42 | — | 2.3 | 41.3 | 84.7 | 2.0 |
| **26** |  | Me | 0.52 | — | 3.3 | — | — | — |
| **27** |  | H | 0.049 | 0.037 | 1.8 | 7.9 | 5.6 | 0.7 |
| **28** |  | H | 0.102 | 0.102 | 2.8 | — | — | — |

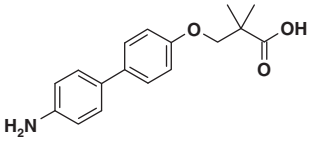
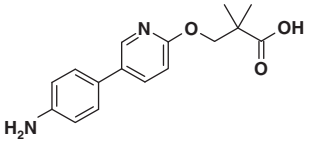
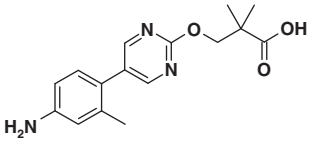
[a] Conducted at Absorption Systems; permeability classification: A–B < $1.0 \times 10^{-6}$ cm/s = low and A–B > $1.0 \times 10^{-6}$ cm/s = high; efflux ratio ⩾3 indicates significant efflux.

additional methylene spacer, coupled with installation of a gem-dimethyl group adjacent to the carbonyl, yielded **14** (Table 2) the most potent analogue identified in the enzymic assay.[12]

Substitution of the heteroatom adjacent to the triazole for a methylene was also accomplished (Scheme 2). Deletion of the heteroatom would be anticipated to increase passive permeability as highlighted above. Starting from 4-nitrobenzhydrazide, the 1,3,4-oxadiazole was assembled. Reaction of this with methylamine trifluoroacetate under forcing conditions generated the *N*-methyl-1,3,4-triazole. Standard conditions were then used to convert the nitro group to the desired amide, and finally to hydrolyse the ester to acid **15**. Pleasingly, the sulphur could be replaced with a methylene and still retain reasonable levels of in vitro potency. Unfortunately, both **14** and **15** showed a considerable drop off in potency when evaluated in the 3T3L1 assay. Permeability data showed **14** and **15** still underwent efflux.

The DGAT1 inhibitors up to this point all contained a carboxylic acid charged at physiological pH, and thus would be expected to be highly solvated. To reduce solvation, amide derivatives of highly potent acid **14** were prepared (Table 3). The amides were typically

**Table 5**
Mutagenicity (Ames activity) of anilines for *Salmonella typhimurium* TA98 in the presence of rat liver S9; $^{*}p < 0.05$

| Compd | Structure | Max fold increase in mutation frequency (TA98 + S9) |
|---|---|---|
| **29** |  | 24* |
| **30** |  | 2.6* |
| **31** |  | No increase |

5 to 10 fold less potent than the parent acid in the DGAT1 enzymic assay. Primary amide **16** displayed high efflux and reduced cell potency over the parent acid, possibly due to increased hydrogen bond donor count. For secondary and tertiary amides, improved passive permeability and cell activity was observed, for example, benzamide **19**, DGAT1 $IC_{50}$ 0.11 μM, 3T3L1 $IC_{50}$ 0.22 μM. For this limited set of examples, cell potency appeared to correlate with efflux ratio. Sub-micromolar cell potency was only obtained with the most lipophilic analogues ($\mathrm{Log}D \geqslant 4.4$). This, together with the significant efflux observed for the majority of analogues led us to seek alternative cores.

In the design of a new core, reducing heteroatom count was an important consideration to mitigate efflux potential. Conformational analysis suggested the central biaryl in triazole **3** was non-planar by virtue of the *N*-methyl group. It was unclear if the triazole nitrogens were participating in any H-bonding interactions, so in order to mimic the non-planar orientation, a methyl was appended to the N-linked phenyl ring. For ease of synthesis, the triazole was replaced with six-membered heterocycles, as outlined in Scheme 3. The central biaryl was constructed first via Suzuki coupling. Following formation of the 2-naphthyl amide, installation of the 2,2-dimethylpropionic acid was conducted either via alkylation or $S_NAr$ reaction, depending on the nature of the aryl ring. The results of this work are shown in Table 4. Although a drop-off in DGAT1 enzymic potency was observed relative to **14**, the compounds showed good inhibition of cellular triglyceride synthesis in 3T3L1 adipocytes. Permeability, as determined by Caco-2 was high, with much reduced efflux. To establish the effect of shape around the biaryl core, analogues **27** and **28** lacking the methyl on the N-linked aromatic were also prepared. Compounds **27** and **28** were found to have enhanced activity compared with the methyl analogues **24** and **26,** respectively.

Concerns about the potential mutagenic activity of the anilines released from metabolic cleavage of the amide bond of these compounds led us to evaluate selected anilines **29**–**31** in the Ames test, against TA98 and TA100 strains ± S9 metabolic activation. The results for strain TA98 + S9 (the most sensitive conditions) are shown in Table 5. The presence of heteroatoms in the distal aromatic appeared to modulate the mutagenic potential of the aniline, with pyrimidine **31** devoid of mutagenic response in this assay (and TA100 ± S9). Shape, lipophilicity and the reactivity of the ultimate nitrenium species have all been reported to modulate the mutagenic activity of aromatic amines and the effect of the presence of the methyl group in **31** cannot be excluded.[13] Subsequent to conducting this study, aniline **30** and an amide derivative was described in a competitor DGAT1 patent.[14]

In summary, a number of strategies were employed to reduce Pgp substrate recognition and increase passive permeability for a series of DGAT1 inhibitors. Starting from a virtual screening hit with poor inhibition of cellular triglyceride synthesis, potent DGAT1 inhibitors with reduced efflux and high permeability were obtained. The mutagenic potential of three prospective aniline metabolites was evaluated in the Ames assay. One aniline was shown to be devoid of mutagenicity.

## Acknowledgments

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7. The calculated value (ACD) of Log*D* at pH 7.4 for **3** was −0.5, more than one log unit higher than measured. For the purposes of this study calculated values were used as a guide.
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11. Compare 2-mercaptoacetic acid, $pK_a$ 3.6 and 3-hydroxypropanoic acid, $pK_a$ 4.5.
12. It was not possible to make the analogue lacking the gem-dimethyl group by this method. Attempted reaction of the sulphone with *tert*-butyl 3-hydroxypropionate afforded the triazol-3-ol, presumably via elimination of *tert*-butyl acrylate from the desired product.
13. For an example of successfully reducing aniline mutagenicity see: Block, M. H.; Boyer, S.; Brailsford, W.; Brittain, D. R.; Carroll, D.; Chapman, S.; Clarke, D. S.; Donald, C. S.; Foote, K. M.; Godfrey, L.; Ladner, A.; Marsham, P. R.; Masters, D. J.; Mee, C. D.; O'Donovan, M. R.; Pease, J. E.; Pickup, A. G.; Rayner, J. W.; Roberts, A.; Schofield, P.; Suleman, A.; Turnbull, A. V. *J. Med. Chem.* **2002**, *45*, 3509.
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