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Solution-Phase Parallel Synthesis of 5-Carboxamido 1-Benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazoles as Activators of Soluble Guanylate Cyclase with Improved Oral Bioavailability

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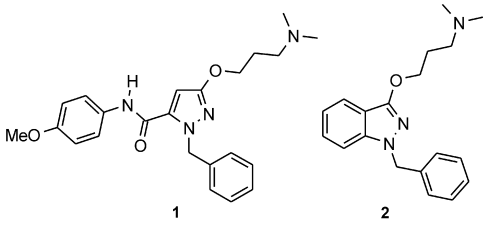
Abstract—A lipophilicity constrained library of 5-carboxamido 1-benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazoles was prepared by solution-phase parallel synthesis with removal of acidic by-products using the strongly basic MP-carbonate resin. Compounds show both activation of soluble guanylate cyclase and inhibition of platelet aggregation. Compound **12** also shows 22% oral bioavailability in rats.

Nitric oxide-induced activation of soluble guanylate cyclase[1] (sGC) is an important cell signalling pathway involved in platelet aggregation, neurotransmission, vasodilation and other physiological processes.[2] Potent and selective activators of sGC are therefore considered as highly desirable both as pharmacological tools to probe the NO-cGMP pathway and as potential therapeutic agents. We have recently reported the discovery of the novel, selective sGC activator and inhibitor of platelet aggregation CFM1571 (**1**) from our initial lead compound, benzydamine (1-benzyl-3-(3-dimethylaminopropyloxy)-1*H*-indazole, **2**).[3] However, pharmacokinetic studies in rats indicate a modest oral bioavailability of 12% for **1**.

The design and solution-phase synthesis of a library of 5-carboxamido 1-benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazole analogues of **1** was carried out with the aim of improving pharmacokinetic properties. The library design was biased using calculated octanol/water partition coefficient constraints to maintain cell penetration and to improve oral bioavailability. Compounds were synthesised via amide couplings and evaluated for sGC activity after purification using a sequestration enabling reagent and strongly basic resin.



Library Design

We have previously reported the evaluation of indazole and pyrazole compounds as activators of sGC in an isolated enzyme assay and inhibition of platelet aggregation assay.[3] Compound lipophilicity analysis using calculated octanol/water partition coefficients showed a general lipophilicity requirement for inhibition of platelet aggregation. These findings were used to create the lower lipophilicity bound of a clogP value greater than 2.0 and an upper bound of 4.0 for the virtual product library.[4] Substituted aryl and heteroaryl primary amines

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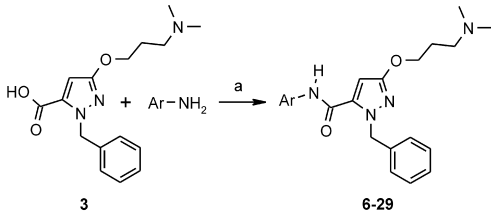
suitable for reaction with the acid **3** via amide couplings were selected from databases of commercially available compounds. Amine reagents with functional groups that were likely to be reactive, toxic or interfere with the reaction were discarded. The virtual product library was enumerated using the Legion module of the Sybyl software suite.[5] Only virtual products with a clogP >2.0 but <4.0 were retained as possible synthetic candidates. A product molecular weight constraint of less than 500 was also applied. Fifty compounds were selected for synthesis from a total of 147. These were selected to encompass the range of clogP values of the product library and to give a good representation of the structural diversity of the candidate reagents.

Chemistry

Automated parallel synthesis of amides is well known in the literature.[6] In this study a solution-phase approach has been adopted utilising solid supported reagent purification techniques. 1-Benzyl-3-(3-di-methylamino-propyloxy)-1*H*-pyrazole-5-carboxylic acid (**3**)[3] was heated in acetonitrile (50 °C, 5 h) with the selected amines in parallel (Scheme 1).[7] *O*-(7-Aza-benzotriazol-1-yl)-*N,N,N,N*-tetramethyluronium hexafluorophosphate (HATU) was used as the coupling reagent. HATU has previously been shown as an effective reagent in peptide coupling chemistry.[8] The resin bound *N,N*-diisopropyl-ethylamine base PS-DIEA[9] (*N,N*-(diisopropyl)amino-methyl polystyrene) was used as a readily removable base.

Subsequent to reaction completion, the sequestration enabling reagent tetrafluorophthalic anhydride (**4**)[10] was added to each mixture. The anhydride reacts with any unreacted amine reagent causing ring opening to afford the acidic product **5** (Scheme 2). This acidic product and any unreacted **3** was subsequently removed using the strongly basic MP-carbonate resin.[9] This treatment also removes the 7-azahydroxybenzotriazole by-product. The resin is a macroporous polystyrene anion exchange resin functionalised with triethylammonium groups. Stirring of the reaction mixtures with an excess of the resin followed by filtration afforded the purified library of amides.

Library analysis was performed by LC–MS.[11] Of the 50 reactions attempted, 24 compounds (**6**–**29**) were found to have a purity of greater than 60%. These compounds were assayed for sGC activation and inhibition of platelet aggregation (Table 1). Yields of the 24 compounds varied between 10 and 70% with the majority greater than 45% as assessed by LC–MS. Reactions that failed were predominantly with hindered amines or amines deactivated with strong electron withdrawing groups. Selected compounds were resynthesised and full analytical data obtained prior to evaluation of platelet aggregation IC_{50}'s and oral pharmacokinetic parameters.[12]



Scheme 1. (a) HATU, PS-DIEA, CH_3CN.

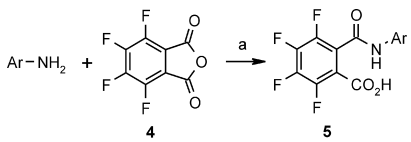
Results

Compounds were assayed for sGC stimulation using an isolated enzyme assay as previously described.[3] sGC was submaximally stimulated with the NO donor DEA/NO (2-(*N,N*-diethylamino)-diazenolate-2-oxide, 300 nM) and cGMP accumulation was evaluated for each compound at a single concentration of 1 μM. The cGMP produced by each compound was expressed as a ratio between the percentage of the DEA/NO response for the compound and that for **2** (EC_{50} 1.02 ± 0.26 μM). Compounds were also evaluated for inhibition of collagen stimulated platelet aggregation at 10 μM. Results are reported in Table 1.

It is clearly seen from the relative enzyme activity (REA) data that many of the compounds show a similar level of activation to that of benzydamine (**2**). The most active compound in the enzyme assay was **22** (REA 1.31), which shows greater activity than both **1** and **2**. However, **22** shows a decrease in platelet activity upon comparison with compounds such as **11** and **12**. No clear SAR trends could be deduced due to the structural diversity of the amide substituent. Furthermore the absence of significant variation in the enzyme data suggests that the amide substituent may not participate in a direct interaction with the enzyme. Therefore modification in this region can be utilised to optimise the pharmacokinetic profile. There was no clear correlation between platelet inhibition data and lipophilicity, although the more lipophilic compounds tend to have a greater likelihood of showing $>70\%$ inhibition of aggregation.

Four compounds were selected and resynthesised to analytical purity for evaluation of platelet aggregation IC_{50}'s and for oral pharmacokinetics in rats (Table 2).[3] The platelet IC_{50} data show that the four compounds are essentially equipotent with compound **1**. However, for compounds **10**, **11** and **12**, there is an improvement in pharmacokinetic profile. Notably, **12** shows a 10% increase in oral bioavailability (F) over **1** and an increased time to maximal concentration of 7.3 h.

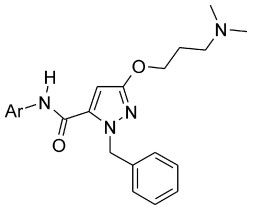
In conclusion, a lipophilicity constrained library of 5-carboxamido 1-benzyl-3-(3-dimethylaminopropyl-

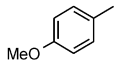
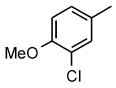
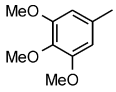
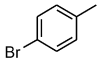
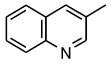
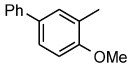
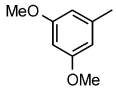
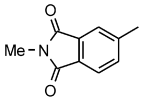
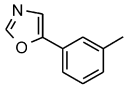
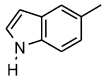
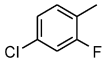
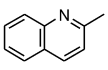
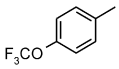
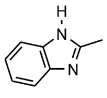
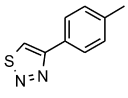


Scheme 2. (a) CH_3CN, 18 h.

oxy)-1*H*-pyrazoles was synthesised using amide coupling chemistry and resin-based purification. Compounds were evaluated for activation of soluble guanylate cyclase and inhibition of platelet aggregation. Compound **12** has been identified as a potent activator of soluble guanylate cyclase and inhibitor of platelet aggregation, which shows improved oral bioavailability over compound **1** (CFM1571).

Table 1. Activity data for 5-carboxamido 1-benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazoles



Ar					
Compound	**1**[a]	**6**	**7**	**8**	**9**
LC–MS purity		80%	88%	90%	93%
REA[b]	1.10	0.88	1.02	0.93	0.96
% Inhibition[c]	83%	93%	58%	95%	66%
Ar					
Compound	**10**	**11**	**12**	**13**	**14**
LC–MS purity	92%	87%	98%	90%	93%
REA[b]	0.87	0.91	0.86	0.87	0.72
% Inhibition[c]	90%	95%	95%	95%	87%
Ar					
Compound	**15**	**16**	**17**	**18**	**19**
LC–MS purity	65%	90%	66%	67%	88%
REA[b]	0.81	0.71	0.90	0.91	0.91
% Inhibition[c]	64%	83%	43%	66%	96%
Ar					
Compound	**20**	**21**	**22**	**23**	**24**
LC–MS purity	93%	82%	92%	88%	98%
REA[b]	0.97	1.06	1.31	0.93	0.74
% Inhibition[c]	57%	27%	80%	85%	96%
Ar					
Compound	**25**	**26**	**27**	**28**	**29**
LC–MS purity	69%	85%	69%	70%	80%
REA[b]	0.76	0.74	0.71	0.85	0.81
% Inhibition[c]	44%	13%	75%	92%	96%

[a]Compound **1** evaluated as an analytically pure sample.
[b]Relative enzyme activity, enzyme activity at 1 μM relative to compound **2**.
[c]% Inhibition of platelet aggregation at 10 μM.

Table 2. Platelet IC_{50} data and pharmacokinetic parameters for sGC activators

Compound	C_{max} (ng/mL)	AUC (ng/h/mL)	t_{max} (h)	F (%)	Platelet IC_{50} (μM)[c]
1[a]	6.4	61.1	2.7	12	2.84±0.44
6[b]	5.4	32.6	4.5	11	2.63±0.20
10[b]	12.1	76.9	5.0	17	3.58±0.41
11[b]	14.3	95.0	5.0	19	3.27±0.02
12[b]	18.0	130.5	7.3	22	2.76±0.55

[a]Dosed at 5 mg/kg po.
[b]Cassette dosing at 2 mg/kg po.
[c]Concentration required to inhibit collagen induced platelet aggregation by 50%. Values are means and standard errors of the means of at least four experiments.

Acknowledgements

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4. Octanol/water partition coefficients were calculated using the clogP program: Leo, A. *Chem. Rev*. **1993**, *93*, 1281, as distributed in Sybyl 6.5 by Tripos Inc., 1699 South Hanley Road, St. Louis, MO, 63144, USA.
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7. General procedure for the parallel synthesis of the amide library: 1-Benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazole-5-carboxylic acid (**3**) (30 mg, 0.1 mmol) was dissolved in anhydrous acetonitrile (1 mL). The aryl amine (0.1 mmol), *N*,*N*-(diisopropyl)aminomethyl polystyrene resin (PS-DIEA), (86.0 mg, 0.3 mmol, loading 3.5 mmol/g) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU) (38.0 mg, 0.1 mmol) were added and the reaction mixture was shaken under nitrogen in anhydrous acetonitrile (4 mL) at 50 °C for 5 h. The reaction mixture was then cooled to rt and tetrafluorophthalic anhydride (65.0 mg, 0.3 mmol) was added and the reaction mixture was shaken for 18 h. Macroporous triethylammonium methylpolystyrene carbonate resin (MP-carbonate; 610 mg, 1.96 mmol, loading 3.18 mmol/g) was then added and the reaction mixture was shaken for a further 48 h. The reactions were then filtered through filter syringes into pre-weighed vials and washed with methanol. The solvent was removed on a vacuum concentrator to afford the product library.
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9. Supported reagents PS-DIEA and MP-carbonate are available from Argonaut Technologies, Switzerland.
10. Parlow, J. J.; Naing, W.; South, M. S.; Flynn, D. L. *Tetrahedron Lett*. **1997**, *38*, 7959.
11. LC–MS analysis was carried out on a Micromass Quattro electrospray LC–mass spectrometer.
12. Compounds were resynthesised according to procedures in ref 3. For **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.60 (d, 1H, J=2.6 Hz), 7.38 (dd, 1H, J=2.6 Hz, J=9.0 Hz), 7.30–7.23 (m, 5H), 6.85 (d, 1H, J=8.7 Hz), 6.12 (s, 1H), 5.62 (s, 2H), 4.15 (t, 2H, J=6.4 Hz), 3.87 (s, 3H), 2.44 (t, 2H, J=7.3 Hz), 2.25 (s, 6H), 1.97–1.88 (m, 2H); MS (EI) m/z 442 [M^+]; $C_{23}H_{27}Cl\ N_4O_3 \cdot 0.5H_2O$; calcd C, 61.12; H, 6.24; N, 12.40; found C, 61.40; H, 6.36; N, 12.31.