Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 72–76
Bioorganic & Medicinal Chemistry Letters

Pyrazole CCK_1 receptor antagonists. Part 1: Solution-phase library synthesis and determination of Free–Wilson additivity

Kelly McClure, Michael Hack, Liming Huang, Clark Sehon, Magda Morton, Lina Li, Terrance D. Barrett, Nigel Shankley and J. Guy Breitenbucher*

Johnson & Johnson Pharmaceutical Research and Development L.L.C., 3210 Merryfield Row, San Diego, CA 92121, USA

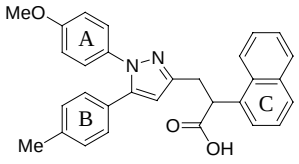
Received 1 August 2005; revised 9 September 2005; accepted 20 September 2005
Available online 19 October 2005

Abstract—High throughput screening revealed compound **1** as a potent antagonist of the CCK_1 receptor. Evaluation of the CCK_1 SAR in a series of these diarylpyrazole antagonists was conducted in a matrix synthesis format revealing additive (Free–Wilson) and non-additive SAR. This use of additive QSAR modeling in conjunction with combinatorial libraries represents a unique approach to the evaluation of SAR interactions between the variables of any combinatorial matrix.

Cholecystokinin (CCK) is an endogenous 33 amino acid peptide hormone first identified by its pharmacological actions in 1928,[1] and later purified and sequenced in 1971.[2] Subsequently, it has been shown that CCK is released in response to food intake and regulates gallbladder contraction, pancreatic enzyme secretion, gastric acid secretion, gastric emptying, duodenal-, and colonic motility. In addition, CCK is abundant in the CNS and is thought to be involved in aspects of nociception, satiety, anxiogenesis, memory, and learning.[3]

The biological actions of CCK are mediated through two G-protein coupled receptors, CCK_1 and CCK_2. CCK's actions on gallbladder contraction, pancreatic enzyme secretion, duodenal motility, and gastric emptying rate appear to be mediated through agonism of the CCK_1 receptor. As a result, a number of CCK_1 antagonists have been evaluated in the clinic for pancreatic disorders, IBS, and biliary colic. Promising clinical results from a phase II trial of constipation dependent IBS with the peptide derived CCK_1 antagonist, dexloxiglumide, encouraged our pursuit of a differentiated non-peptide derived antagonist of CCK_1[4] (Fig. 1).

Compound **1** was identified through a high throughput screening campaign as a potent and selective antagonist



1

Figure 1. Lead CCK_1 antagonist from HTS.

at the CCK_1 receptor ($pK_I = 7.6$). The compound had promising physical properties and constituted a novel chemical scaffold for SAR investigations.[3a]

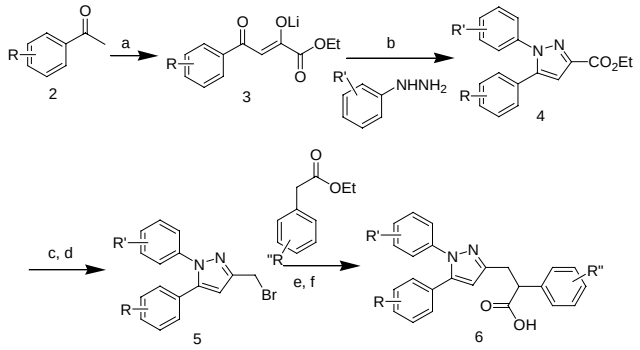
The modular synthesis of compound **1** (Scheme 1) led to the investigation of both solid- and solution-phase library strategies to establish the SAR around this lead. The work on solution-phase libraries is reported here, while the solid-phase approach is reported in the accompanying paper.

In these investigations, a novel method for the evaluation of SAR in combinatorial matrices was discovered. Namely, the quantitative assessment of additive and non-additive relationships in the SAR allowed us to identify potential changes in the binding modes of these antagonists. These methods may prove generally applicable to the evaluation of SAR in a combinatorial matrix of ligands against any biological target.

The solution-phase approach described here was well suited to the simultaneous investigation of the C-ring

Keywords: CCK_1; Cholecystokinin; Combinatorial; Additivity; Pyrazole.
* Corresponding author. Tel.: +1 858 784 3036; e-mail: jbreiten@prdus.jnj.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.09.048



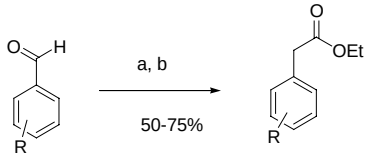
Scheme 1. Reagents and conditions: (a) diethyl oxalate, LiHMDS, ether; (b) THF, TsOH, 40 °C; (c) DIBAL, THF, −78 °C to rt: (d) CBr_4, PPh_3, DCM; (e) NaH, DMF; (f) LiOH, THF, MeOH, H_2O, 50 °C.

and the A- or B-rings of **1**, and was devised for the initial SAR studies (Scheme 1).

Multi-gram quantities of 1,5-diarylpyrazole bromides (**5**) were synthesized using the procedure described by Murray et al.[5] Acetophenones **2** were condensed with diethyl oxalate to afford the lithium salt of diketones **3** in quantitative yield. Treatment of **3** with arylhydrazines provided 1,5-diaryl pyrazoles **4** in 60–90% yield. The selectivity for the formation of the 1,5-pyrazoles over the 1,3-pyrazoles was >10:1 in all cases, owing to the increased electrophilicity of the α-ketoester. Esters **4** could then be readily converted to the bromides **5** (Scheme 1). The final bond connection in the sequence involved alkylation of commercial phenyl acetic acid esters with the pyrazole bromides **5**, and hydrolysis of the resulting ester with LiOH to afford the targeted acids **6**. These final two steps were carried out in a 48-well parallel format in a Bohdan Miniblock, and the compounds were purified by automated reverse-phase preparative HPLC.

For cases where the phenyl acetic acids were not commercially available, the procedure in Scheme 2 was used.[6]

The synthetic scheme used to access these analogs allowed for the simultaneous evaluation of variations of the naphthyl group (Ar^3) and of the diarylpyrazole substituents (Ar^1 or Ar^2). The first library was composed of the building blocks depicted in Figure 2 with variables at positions Ar^2 and Ar^3. The CCK_1 binding results of the first library are reported in graphical form in Figure 3.[7] Depiction of the results in the bar-graph format allows for the visualization of trends in the data relative to the structural changes made. The *X* (Ar^2) and *Y* (Ar^3)



Scheme 2. Reagents and conditions: (a) thiomethyl-dimethyl sulfoxide, Triton B, methanol, dioxane, 80 °C; (b) HCl, EtOH, rt, 12 h.

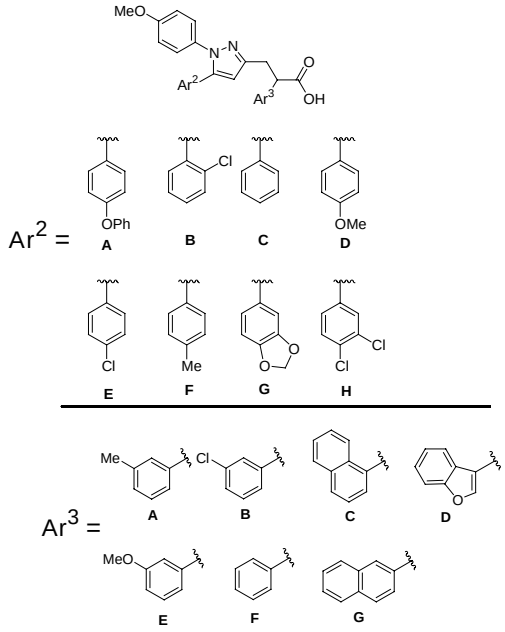


Figure 2. Input variables for library 1.

axes represent the structural changes made to each variable fragment of the series. The *Z* axis shows binding affinity expressed as the negative log of the affinity constant (pK_I). The white bar at position Ar^2_F–Ar^3_C represents the activity of the original lead compound **1**.

The library provided compounds which spanned a range of activity from pK_I = 6.2–8.1. The nine compounds not represented in the graph are compounds that were not tested due to difficulties in synthesis and/or purification. The library above was then analyzed using an additive QSAR method.

Additive models of SAR were first described by Free and Wilson in 1964 and later modified by Fujita and Ban in 1971.[8,9] These models are generally used to predict the activity of all compounds in a matrix when a cross-section of analogs is synthesized. The assumption made is that one variable in the structure does not effect the binding or conformation of the second variable. However, in many documented cases this assumption is invalid, thus limiting the predictive power of additive models.[8] Interestingly despite this knowledge many medicinal chemistry papers continue to report uni-dimensional analoging. In these cases, chemists seem prepared to accept the risks of missing key pieces of SAR due to non-additive behavior, or are perhaps unaware of the limitations of the additive assumption.

Fortunately, with the advent of combinatorial methods, a full matrix of compounds can be readily accessed synthetically. In this situation, the presence of non-additive relationships in the full matrix can suggest either a different binding mode in the biological target or direct interactions between different parts of a ligand.

Traditionally, the Fujita–Ban equation[9] has been used to model non-additive effects in libraries. Our case is

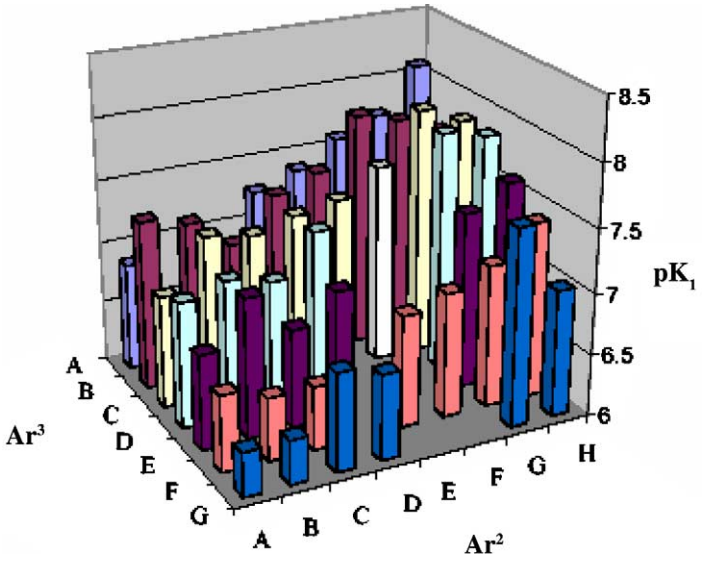


Figure 3. CCK_1 binding data from library 1.

slightly different in that we do not have an unsubstituted compound to use as a reference. Therefore, we use the following modified form of the Fujita–Ban equation:

$$pK_I = \mu\Sigma b_i + \Sigma b_j. \quad (1)$$

In this equation, μ represents the activity associated with an arbitrary compound in a two-dimensional matrix of compounds, such as the one shown in Figure 3. This compound serves as a reference. b_i represents the change in activity associated with moving from the reference row to a different row i in the matrix (in this case, associated with changing the group at Ar^2), and b_j represents the change in activity associated with moving from the reference column to a different column j (in this case, changing the group at position Ar^3). Thus, the activity of any compound in the matrix is represented by the sum of three numbers: the reference, plus a row value (0 if the compound is in the reference row), and a column value (0 if the compound is in the reference column). The assumption in this model is that the activity of column i does not depend on the choice of row j. Mathematically, this is the additive assumption. For an $N \times M$ matrix of compounds, this gives $N + M - 1$ parameters to fit using a least-squares procedure. Note that by evaluating the entire matrix the highest possible confidence is achieved in the fitting of the variables of the additivity model. Note also that a change in the choice of the reference compound does not affect the quality of the fit (given by r^2), but will affect the value of the individual variables μ, b_i, and b_j.

Figure 4 shows a plot of the activities as predicted by Eq. 1 against the experimentally determined ones. In this case, r^2 is 0.91 and the RMS error is 0.16. This is statistically equivalent to the experimental uncertainty of the assay, which has an average standard error in three replicate determinations of 0.17 log units.

Thus, the additive model seems to apply in this case; there do not appear to be special cases where particular

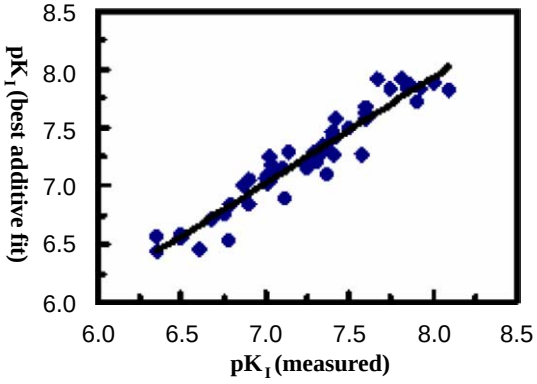


Figure 4. Library 1, plot of actual pK_I's against the calculated pK_I's as determined by the best least-squares fit to Eq. 1.

substituents in one variable alter the SAR of variables in the second dimension. Therefore, our assumption in this matrix is that all the compounds probably bind in a similar binding mode to the receptor.

A second library was then synthesized where Ar^2 was held constant, and Ar^1 and Ar^3 were altered simultaneously utilizing the same synthetic method used for library 1. The inputs for this library are shown in Figure 5. The CCK_1 binding results for the second library are presented in graphical form in Figure 6. These results were analyzed in the same fashion as the first library and the resulting least-squares fit to the additive model is plotted against the experimentally determined pK_I's in Figure 7. In this case, the fit is significantly worse than that obtained from library 1 ($r^2 = 0.75$, RMS error = 0.25).

Figure 7 illustrates distinct outliers that do not appear to conform to the additive hypothesis. Interestingly, these outliers are seen only in the series where Ar^1 = benzyl (orange bars, Ar^1_F). If we leave out the Ar^1_F series of the matrix and recalculate the

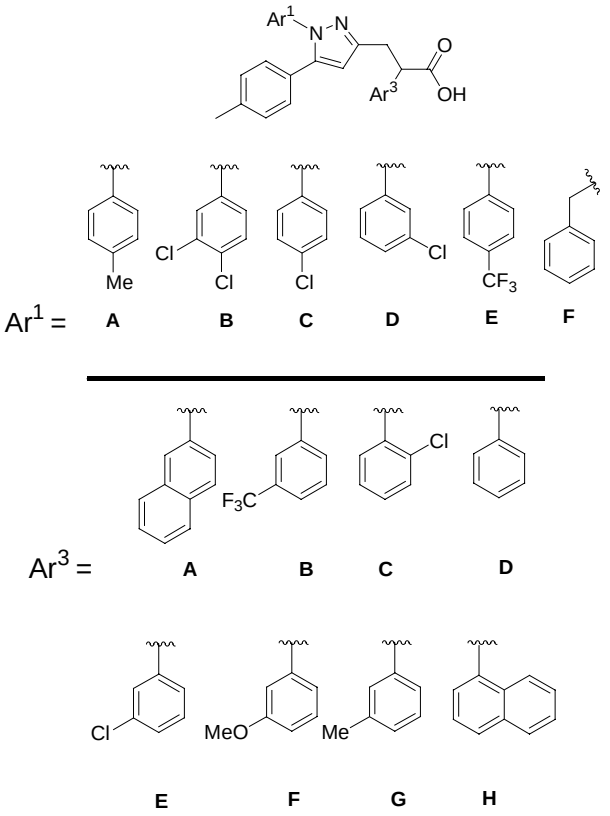


Figure 5. Input variables for library 2.

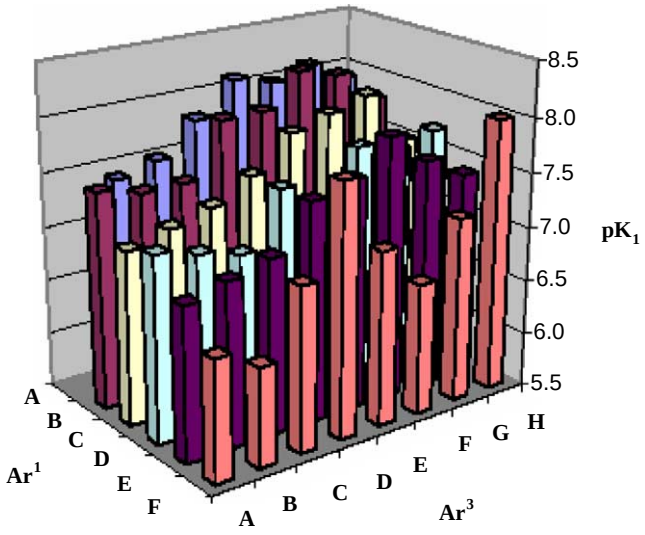


Figure 6. CCK_1 binding data from library 2.

least-squares fit of the remaining data and re-plot the data against the actual data in the new matrix, the result is given in Figure 8. Figure 8 demonstrates conformance to the additive model $r^2 = 0.9$, RMS error = 0.13).

One interpretation of this result is that in an effort to maintain an optimal alignment of the A- and B-rings in the receptor, the Ar^1_F series of compounds must adjust their binding modes slightly, effectively reorienting Ar^3

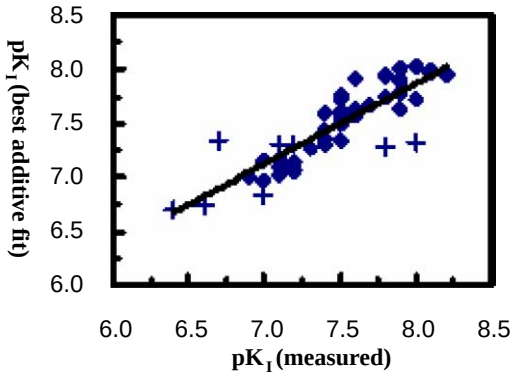


Figure 7. Library 2, plot of actual pK_I's against the calculated pK_I's as determined by the best least-squares fit to Eq. 1. + symbol represents the data from row Ar^1_F.

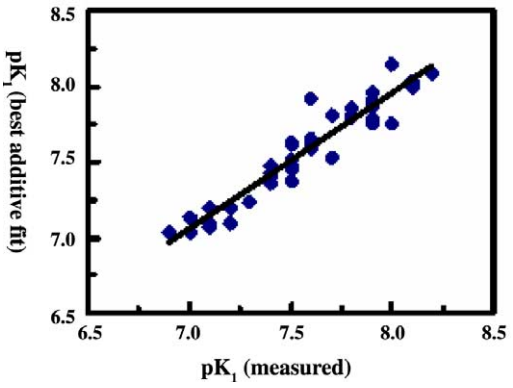


Figure 8. Library 2, plot of actual pK_I's against the calculated pK_I's as determined by the best least-squares fit to Eq. 1, omitting data from row Ar^1_F.

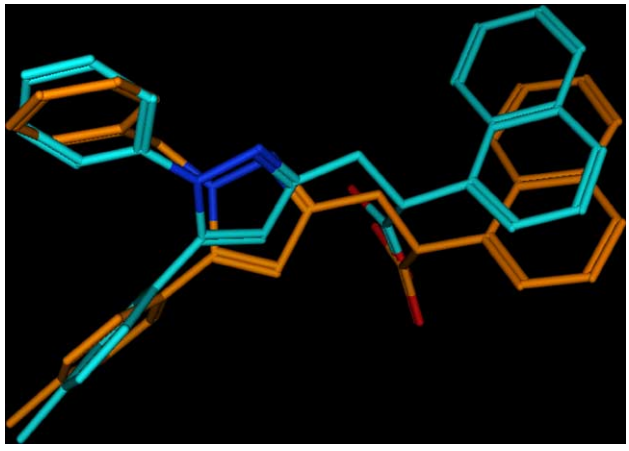


Figure 9. Superposition of aryl and benzyl pyrazole.

relative to the aryl pyrazole. An example of a superposition of an aryl and benzyl pyrazole is shown in Figure 9.

This is only required in the case where the additional methylene in the benzylic series extends the distance

between the pyrazole ring and Ar^1. This adjustment in the way the series binds is manifested in a change in the SAR of the Ar^3 substituent. This model is in agreement with the models previously reported by Varanavas et al.[10] which suggests a pharmacophore containing two aryl binding elements spaced by a distance of 9.5 Å. In fact, recent modeling suggests that the distance and orientation of the key lipophilic components required for binding in an anthranilic acid series of antagonists were critical for binding and may supply a similar driving force for non-additive behavior in this instance as well.[11]

CCK_1 antagonists have potential therapeutic utility in IBS, pancreatitis, and other GI disorders. Here we describe a novel class of potent pyrazole-based CCK_1 antagonists. The SAR was evaluated using a matrix synthesis approach allowing for the quantitative determination of additive relationships between substituents in the matrix. In this case, all but one series complied with an additive SAR model. In the case of the substituent that was non-additive, we propose a change in binding mode that allows this series to comply with previously published CCK_1 binding models. This is the first case we are aware of combining combinatorial libraries with additive QSAR modeling to obtain a better understanding of interactions which effect binding modes within a matrix. Further in vivo data and SAR on these compounds will be presented in future publications.

Acknowledgments

We thank Jiejun Wu and Heather McAllister for analytical chemistry support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.09.048.

References and notes

1. Ivy, A. C.; Oldberg, E. *Am. J. Physiol.* **1928**, *86*, 599.
2. Mutt, V.; Jorpes, E. *Biochem. J.* **1971**, *125*, 57.
3. For recent comprehensive reviews of CCK phamacology and medicinal chemistry see: (a) Herranz, R. *Med. Res. Rev.* **2003**, *23*, 559; (b) Tullio, P.; Delarge, J.; Pirotte, B. *Expert Opin. Investig. Drugs* **2000**, *9*, 129.
4. Varga, G. *Curr. Opin. Investig. Drugs* **2002**, *3*, 621.
5. Murray, W. V.; Hadden, S. K.; Wachter, M. P. *J. Heterocyclic Chem.* **1990**, *27*(7), 1933.
6. Eyrolles, L.; Kawachi, E.; Kagechika, H.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull.* **1994**, *42*(12), 2575.
7. Biological methods and tabular data of CCK_1 binding are contained in supplemental materials. All compounds were purified by preparative HPLC and identified by H^1 NMR and MS. All compounds were tested as the racemates.
8. Free, S. M.; Wilson, J. W. *J. Med. Chem.* **1964**, *7*, 395.
9. Fujita, T.; Ban, T. *J. Med. Chem.* **1971**, *14*, 148.
10. Varanavas, A.; Lassiani, L.; Valentina, V.; Berti, F.; Mennuni, L.; Makovec, F. *Bioorg. Med. Chem.* **2003**, *11*, 741; Howbert, J. J.; Lobb, K. L.; Britton, T. C.; Mason, N. R.; Burns, R. F. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 875; Agrawal, V. K.; Sharma, R.; Khadikar, P. V. *Bioorg. Med. Chem.* **2002**, *10*; Archer-Lahlou, E.; Tikhonova, I.; Escrieut, C.; Dufresne, M.; Seva, C.; Pradayrol, L.; Moroder, L.; Maigret, B.; Fourmy, D. *J. Med. Chem.* **2005**, *48*, 180.
11. Varnavas, A.; Lassiani, L.; Valenta, V.; Berti, F.; Tontini, A.; Mennuni, L.; Makovec, F. *Euro. J. Med. Chem.* **2004**, *39*, 85.