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SAR studies of 1,5-diarylpyrazole-based CCK_1 receptor antagonists

Laurent Gomez,* Michael D. Hack, Kelly McClure, Clark Sehon, Liming Huang, 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

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Abstract—A high throughput screening campaign revealed compound **1** as a potent antagonist of the human CCK_1 receptor. Here, we report the syntheses and SAR studies of 1,5-diarylpyrazole analogs with various structural modifications of the alkane side chain of the molecule. The difference in affinity between the two enantiomers for the CCK_1 receptor and the flexible nature of the linker led to the design of constrained analogs with increased potency.

Cholecystokinin (CCK) was originally identified in the gastrointestinal tract where it was shown to mediate contraction of the gallbladder.[1] Subsequent purification and peptide sequencing of a cholecystokinin containing extract from the hog intestinal mucosa[2] revealed that CCK is a 33 amino-acid peptide identical to the hormone implicated in pancreatic enzyme secretion.[3] The physiological effects of CCK are mediated by two G-protein coupled receptors, the CCK_1 (formerly, CCK-A) and CCK_2 (formerly, gastrin/CCK-B) receptors, for which it expresses similar affinity and potency. CCK has several activities in addition to the two that led to its discovery. It is reported to cause satiety, inhibit gastric emptying and acid secretion by indirect mechanisms, and appears to play a major role in peristalsis. Various selective CCK_1 receptor antagonists have been examined in the clinic for potential application in irritable bowel syndrome (IBS), non-ulcer dyspepsia, biliary colic, chronic constipation, and pancreatic cancer.[4]

Recently, we reported the results of a high throughput screening campaign leading to the identification of compound **1** as a novel pyrazole-based CCK_1 receptor antagonist (Fig. 1).[5] Previous communications from our laboratories disclosed the concomitant influence of the substitution around three aromatic rings on activity at the CCK_1 receptor.[5] In this paper, we describe the syntheses and SAR studies that were undertaken to evaluate various structural modifications of the linker portion of the molecule.

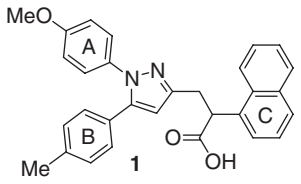

Figure 1. Lead CCK_1 antagonist identified from HTS.

Initial investigations were aimed at studying the influence of the substitution around the C-ring on activity at the CCK_1 receptor. It is important to note that we had previously quantitatively determined additive relationships between both the A- and C-ring and B- and C-ring. Therefore, a one-dimensional optimization of the C-ring substitution was undertaken knowing that the results could be applied to a combinatorial array of A- and B-rings. A solution-phase synthesis was adopted using the procedure described by Murray et al. (Scheme 1).[6] This approach has the advantage of introducing diversity at the last stage of the synthesis allowing for rapid access to various analogs. Condensation of substituted acetophenone **2** with diethyl oxalate under basic conditions afforded the desired β-ketoester as the lithium salt **3** in quantitative yield. The pyrazole ring formation was accomplished using 4-methoxyphenylhydrazine to yield the 1,5-diarylpyrazole in good yield as a 10:1 mixture of regioisomers in favor of compound **4**. The resulting ester was then reduced in the presence of DIBAL-H to afford the desired

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* Corresponding author. Tel.: +1 858 320 3383; fax: +1 858 450 2089; e-mail: lgomez4@prdus.jnj.com

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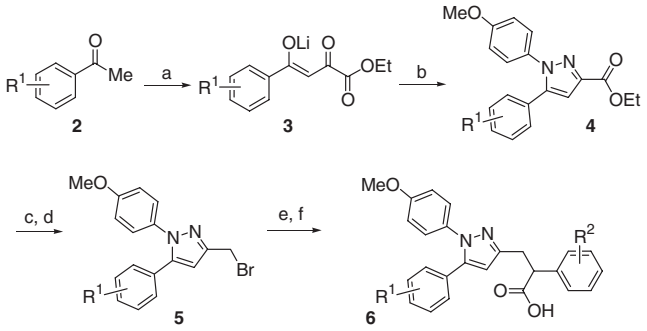


Scheme 1. Reagents and conditions: (a) diethyl oxalate, LiHMDS, ether, −78 °C to rt; (b) 4-methoxyphenylhydrazine, THF, TsOH, 40 °C; (c) DIBAL, THF, −78 °C to rt; (d) CBr_4, PPh_3, DCM, 0 °C to rt; (e) NaH, phenyl acetates, DMF, 0 °C to rt; (f) LiOH, THF, MeOH, H_2O, 50 °C.

alcohol in quantitative yield. A subsequent bromination of the primary alcohol using CBr_4/PPh_3 yielded the key intermediate **5** in excellent yield. The final steps of the sequence were run in parallel and consisted in alkylating compound **5** using NaH and various phenyl acetic acid esters followed by hydrolysis of the resulting esters. The final compounds were purified by automated reverse-phase preparative HPLC.

The binding data (pK_i) of a subset of compounds are shown in graphical form in Figure 2. Analysis of this data set shows similar trends across the two series suggesting an additive relationship between both the B- and C-rings. This investigation shows that meta-substituted phenyl rings consistently display high affinity for the CCK_1 receptor. Altering the positioning of the substituent around the ring to an ortho or para position leads to a 10-fold decrease in activity. This result was further supported by the low affinity observed with the naphthyl substitution.

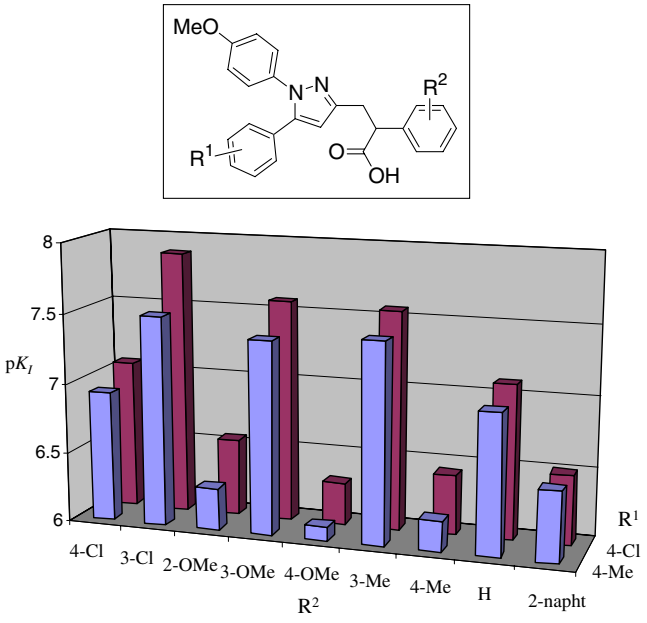
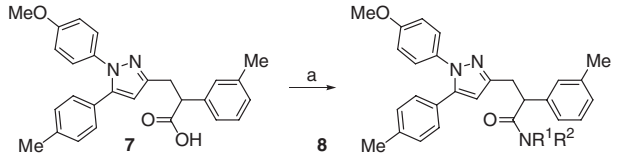


Figure 2. CCK_1 binding data from C-ring substitutions.

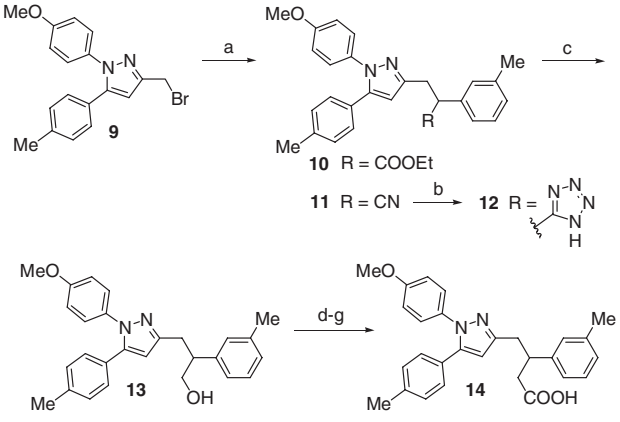
After studying the effect of C-ring substitutions, efforts were made toward evaluating the role of the pendant carboxylic acid. A series of hydrogen-bond donors and acceptors were investigated. The synthetic route used to access analogs represented by **8** involved activation of the carboxylic acid followed by amide-bond formation (Scheme 2). Alternatively, compounds **10** and **11** were synthesized by alkylation of the pyrazole bromide **9** with the desired nucleophiles (Scheme 3). A subsequent reduction of compound **10** using DIBAL-H afforded the corresponding primary alcohol **13** in good yield. Bromination of compound **13** followed by cyanation using sodium cyanide gave the desired product in 65% yield. Hydrolysis of the corresponding nitrile under acidic conditions afforded compound **14** in 82% yield. The binding data (pK_i) are plotted in Figure 3.

Analysis of the data suggests that highly ionized groups, such as carboxylic acids or tetrazoles, are well tolerated and lead to compounds with pK_i values ranging from 7.6 to 8.1. However, incorporation of less acidic functionalities such as primary alcohols results in analogs with reduced affinity. Primary, secondary or bulky tertiary amides are not tolerated. Interestingly, a small tertiary amide (hydrogen-bond acceptor only) displayed similar activity as the carboxylic acid. However, the presence of the carboxylic acid functionality greatly improved the solubility properties of the resulting analogs and was therefore selected for further studies.

The binding data reported thus far have been for the racemic mixture. It became important to evaluate the sensitivity of the CCK_1 receptor to the absolute stereo-



Scheme 2. Reagents and condition: (a) NHR^1R^2, EDC, HOBT, DMF, rt.



Scheme 3. Reagents and conditions: (a) NaH, $ArCH_2CO_2Et$ or $ArCH_2CN$, DMF, 0 °C to rt; (b) NaN_3, NH_4Cl, DMF, 100 °C; (c) DIBAL, THF, −78 °C to rt; (d) PBr_3, DCM, 0 °C to rt; (e) NaCN, DMF, 100 °C; (f) H_2SO_4, MeOH, reflux; (g) LiOH, THF, MeOH, H_2O, 50 °C.

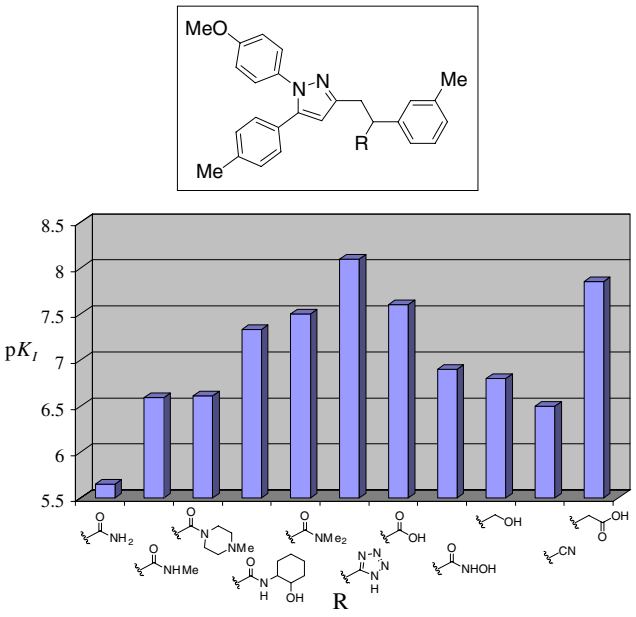
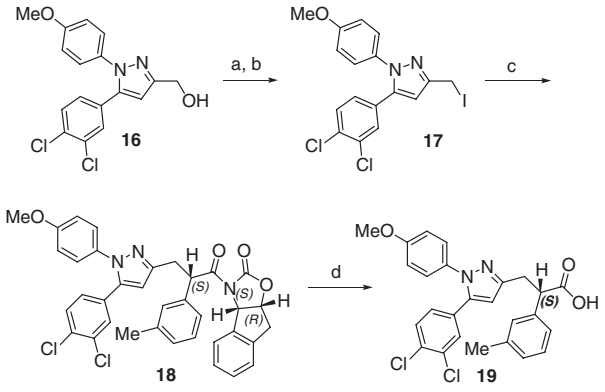


Figure 3. CCK_1 binding data of carboxylic acid replacements.

chemistry at the chiral center. To accomplish this investigation, an initial enantioselective synthesis of pyrazole was developed using the Evans asymmetric alkylation procedure as shown in Scheme 4.[7] Iodination of compound **16** was accomplished in two steps, which involved activation of the primary alcohol using MsCl followed by a Finkelstein reaction to provide the pyrazole iodide in 97% overall yield. The final bond connection of the sequence involved alkylation of **15** with the pyrazole iodide using NaHMDS as a base which afforded compound **18** in 83% yield. The optimal conditions for the release of the chiral auxiliary required the use of hydrogen peroxide under basic conditions. Under these conditions, the desired *S*-enantiomer **19** was isolated in 82% yield (99% ee) with modest recovery of the chiral auxiliary (66%, unoptimized). The preparation of compound **15** involved alkylation of *S*-(3a-*cis*)-(-)-3,3a,8,8a-Tetrahydro-2*H*-indeno[1,2-*d*]-oxazol-2-one with *m*-tolyl-acetic acid using Mukaiyama's reagent under basic conditions to afford the desired product **15** in 74% yield (Scheme 5). Alternatively, the *R*-enantiomer could be obtained using a similar route starting from *R*-(3a-*cis*)-(+)-3,3a,8,8a-Tetrahydro-2*H*-indeno[1,2-*d*]-oxazol-2-one.

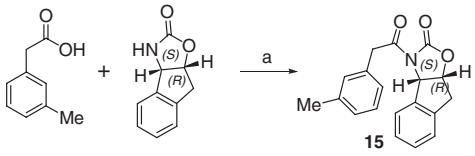


Scheme 4. Reagents and conditions: (a) CH_3SO_2Cl, TEA, THF, rt; (b) NaI, acetone, reflux; (c) **15**, NaHMDS, THF, −78 °C to rt; (d) 30% H_2O_2, LiOH, H_2O, 0 °C to rt.

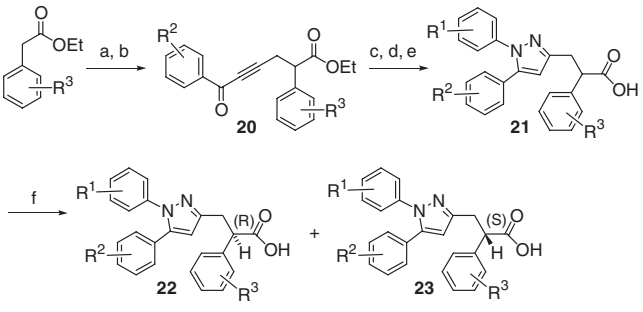
Alternatively, the pure enantiomers could be isolated by chiral chromatography using a racemic synthesis as shown in Scheme 6. Alkylation of phenyl acetates with propargyl bromide under basic conditions followed by a Sonogashira coupling reaction under standard conditions afforded the desired ynones, **20**, in 40–60% overall yields. A subsequent pyrazole ring formation was accomplished using substituted phenyl hydrazines. It was found that optimal dehydration of the cyclized intermediate required the addition of *p*-TsOH to afford the desired 1,5-substituted pyrazoles **21** in 75–80% yield along with a small amount of the 1,3-substituted regioisomer (10–15%, not shown). Hydrolysis of the ester followed by chiral separation using supercritical-fluid chromatography (SFC) gave pure enantiomers **22** and **23** in 80% yield. It should be noted that the absolute configuration of the products was assigned by comparing the analytical data with products isolated from the enantioselective synthesis discussed previously.

The binding data of selected compounds are summarized in Figure 4. The data suggest a strong preference for the *S*-enantiomer which consistently displays about 1.5 log unit increase in binding affinity at the CCK_1 receptor over the *R*-isomer.

Devazepide ($pK_i = 8.74$)[8] and Lintitript ($pIC_{50} = 9.25$) are two well-known CCK_1 antagonists. There are visual similarities between these two antagonists, in particular, the indole amide moiety and pendant phenyl ring. There are also similarities between our compounds and these



Scheme 5. Reagents and condition: (a) 2-chloro-1-methylpyridium iodide, TEA, DMAP, DCM, 0 °C to rt.



Scheme 6. Reagents and conditions: (a) LDA, propargyl bromide, −78 °C to rt; (b) ArCOCl, *N*-methylmorpholine, $PdCl_2(PPh_3)_2$, CuI, THF/toluene, rt; (c) arylhydrazine, THF, rt; (d) *p*-TsOH, THF, rt; (e) LiOH, THF/MeOH/H_2O, 35 °C; (f) SFC, AD column.

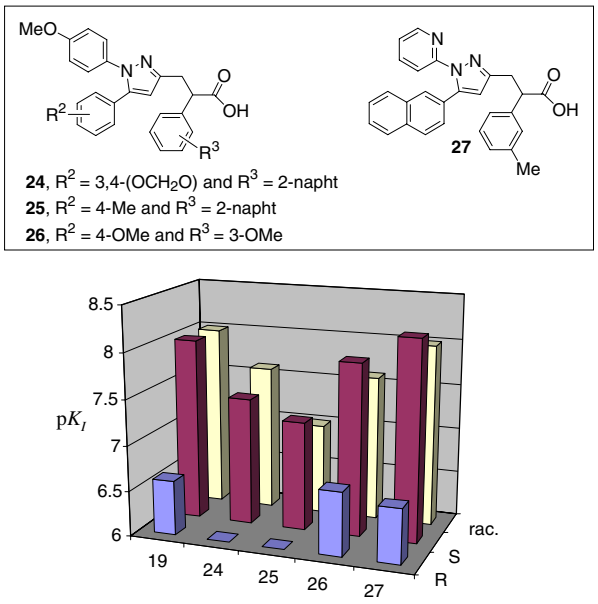


Figure 4. CCK_1 binding data: influence of the chiral center.

antagonists, especially with Devazepide, which appears to array its aromatic rings in a similar way. A three-dimensional molecular alignment of Devazepide, Lintitript, and one of our antagonists is shown in Figure 5.[9]

The figure shows good overlap between the three lipophilic aromatic rings as well as the carbonyl groups of Devazepide and Lintitript and the acid moiety of our pyrazole alkane, suggesting a similar binding mode for all three classes of compounds. More importantly, it appears that the flexible linker of the pyrazole overlays with the more rigid amide functionality of both literature CCK_1 antagonists. These observations led us to propose another question regarding the geometry of the linker: can we incorporate the rigidity found in Devazepide and Lintitript into our side chain and perhaps lock it into an active conformation, thereby

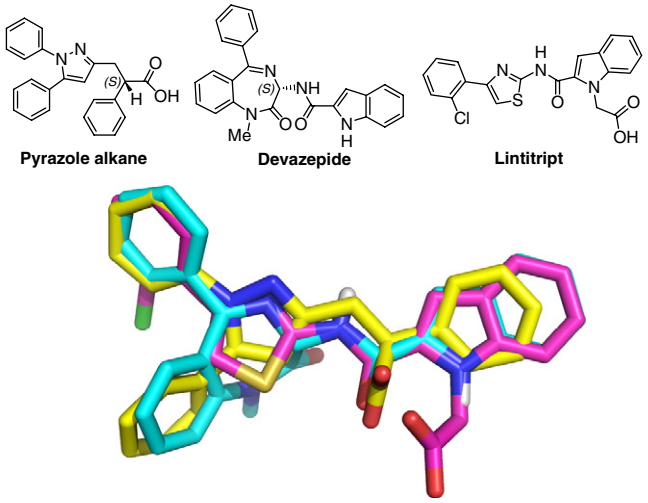


Figure 5. Molecular alignment of a pyrazole alkane (yellow) with known CCK_1 antagonists Devazepide (blue) and Lintitript (magenta).

increasing the potency of our CCK_1 receptor antagonists? An alignment of such a constrained analog with Devazepide and Lintitript is shown in Figure 6. It can be seen that the constrained linker does not visually disrupt the alignment between the molecules. The synthetic route to access this series is shown in Scheme 7.

Oxidation of 1,5-diarylpyrazole alcohols using Dess–Martin periodinane followed by a Perkin condensation of the resulting aldehydes with phenyl acetic acids afforded the desired alkenes **29** as a single *E*-isomer in 85–90% yields. It was found that upon exposure to intense focused light in a quartz tube, this material equilibrated to a thermodynamic mixture of *E*- and *Z*-isomers in 1:1 ratio after 12 h. Both isomers were easily separated by column chromatography to afford **29** and **30** in 70% combined yields. The geometry of the olefin was assigned using 2D NMR spectroscopy and nOe studies. Also, it should be noted that photochemical stability

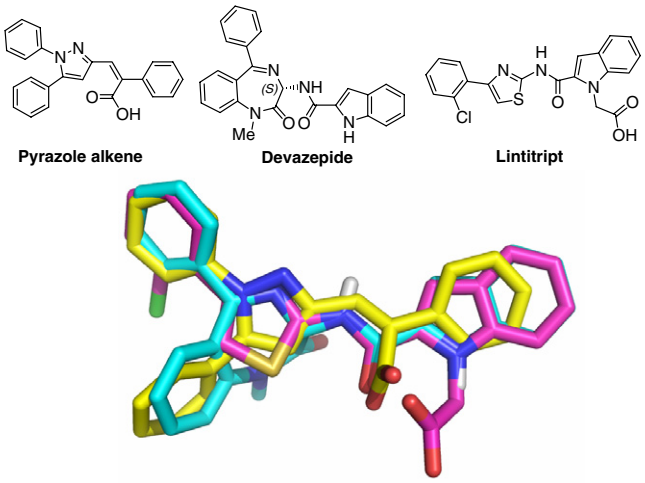
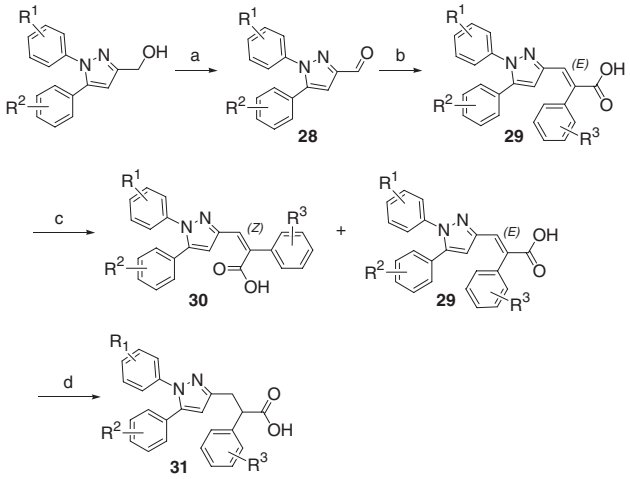


Figure 6. Molecular alignment of a pyrazole alkene (yellow) with known CCK_1 antagonists. Devazepide (blue) and Lintitript (magenta).



Scheme 7. Reagents and conditions: (a) Dess–Martin periodinane, DCM, rt; (b) $ArCH_2COOH$, NEt_3, Ac_2O; (c) uv light, $CHCl_3$, rt; (d) *p*-$TosNHNH_2$, NaOAc, EtOH, 100 °C.

studies were conducted on both the *E*- and *Z*-isomers. It was found that the compounds were extremely stable when stored as dry powder and subjected to intense focused light for several days. However, when stored in solution (0.01 and 1 mg/mL at pH 11) the compounds were moderately stable with approximately 80% recovery after 3 days. A subsequent reduction of the alkene functionality could be performed using *p*-tosyl hydrazine under basic conditions to obtain the corresponding alkane **31** in 75% yield.

A direct comparison of the binding affinity for both the *E*- and *Z*-isomers clearly indicates that the relative orientation of the carboxylic acid and the aromatic group with respect to the pyrazole ring is crucial for good interaction with key residues in the active site, consistent with the alignment illustrated in Figure 6. Regardless of substitutions, analogs bearing the *Z*-configured double bond consistently display greater activity than their *E*-isomer counterparts (Fig. 7).[10] More importantly, when compared to the fully saturated linker (racemic alkane), constrained side-chain analogs with *Z*-configuration display greater activity as shown in Figure 8. Even considering the small difference in activity between the racemate and the pure *S*-enantiomer (which does not exceed 0.3 log unit), the improved activity is significant and consistent across a set of five compounds. The amplitude of the gain in activity (0.3–0.6 log unit) suggests that reducing the overall entropy of the ligands by constraining the flexible linker did have the expected effect. These results are consistent with calculations conducted on the reduction of entropy of a single carbon–carbon bond. The gain was estimated to be about 0.4 kcal/mol, which corresponds to approximately 0.3 log unit of activity at 298 K.[11] It appears that the increase in activity is mainly a reflection of the gain in entropy, which suggests that both the alkane and alkene analogs adopt a similar binding mode.

In conclusion, investigations presented in this article show a clear sensitivity to the chiral center present on

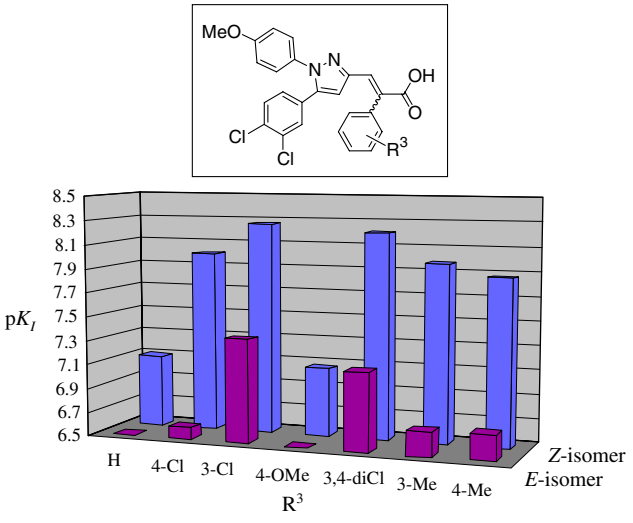


Figure 7. CCK_1 binding data: influence of the geometry of the double bond.

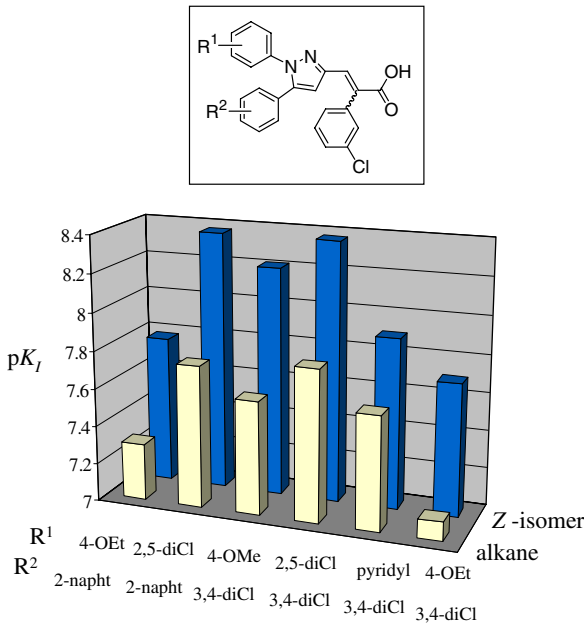


Figure 8. CCK_1 binding data: comparison between alkane and *Z*-isomer.

the molecule with a strong preference for *S*-enantiomers. Further studies showed that the flexible linker could successfully be replaced by a more rigid alkene which resulted in an increase affinity for the CCK_1 receptor. The chemistry developed to access both *E*- and *Z*-isomers involved equilibration of *E*-isomers using intense focused light. This process allowed us to rapidly access a large numbers of analogs. In addition, rat pharmacokinetic studies conducted on compound **19** show excellent oral bioavailability (2 μmol/kg; $\%F = 100$) and good half-life ($t_{1/2} = 5.6 \pm 0.7$ h). These potent pyrazole-based CCK_1 receptor antagonists,[12] with good pharmacokinetic properties and a good selectivity profile over other targets,[13] represent a novel class of compounds for the potential treatment of IBS, pancreatitis and other GI disorders.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.093.

References and notes

1. Ivy, A. C.; Olderg, E. *Am. J. Physiol.* **1928**, *86*, 599.
2. Jorpes, E.; Mutt, V. *Acta Physiol. Scand.* **1966**, *66*, 196.

3. Harper, A. A.; Raper, H. S. *J. Physiol.* **1943**, *102*, 115.
4. (a) Varga, G. *Curr. Opin. Investig. Drugs* **2002**, *3*, 621; (b) Herranz, R. *Med. Res. Rev.* **2003**, *23*, 559; (c) Varga, G.; Balint, A.; Burghardt, B.; D'Amato, M. *Br. J. Pharmacol.* **2004**, *141*, 1275; (d) Peter, S. A. S.; D'Amato, M.; Beglinger, C. *Dig. Dis.* **2006**, *24*, 70.
5. McClure, K.; Hack, M.; Huang, L.; Sehon, C.; Morton, M.; Li, L.; Barrett, T. D.; Shankley, N.; Breitenbucher, J. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 72; Sehon, C.; McClure, K.; Hack, M.; Morton, M.; Gomez, L.; Li, L.; Barrett, T. D.; Shankley, N.; Breitenbucher, J. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 77.
6. Murray, W. V.; Hadden, S. K.; Wachter, M. P. *J. Heterocycl. Chem.* **1990**, *27*, 1933.
7. (a) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1981**, *104*, 1737; (b) Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev.* **1996**, *96*, 835.
8. Data generated using our assay conditions. For details, see: Morton, M. F.; Pyati, J.; Dai, H.; Li, L.; Moreno, V.; Shankley, N. P. *Br. J. Pharmacol.* **2005**, *145*, 374.
9. MOE (The Molecular Operating Environment) Version 2006.08, software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A 2R7. ⟨http://www.chemcomp.com⟩.
10. It should be noted that 3,4-diCl substitutions on the B-ring display comparable activities than 4-Cl and 4-Me (see supplementary material).
11. Lazardis, T. *Curr. Org. Chem.* **2002**, *6*, 1319.
12. For in vivo and in vitro pharmacology of 1,5-diarylpyrazole-based CCK_1 receptor antagonists, see: Morton, M. F.; Barrett, T.; Yan, W.; Freedman, J. M.; Lagaud, G.; Prendergast, C. E.; Moreno, V.; Pyati, J.; Figueroa, K.; Li, L.; Wu, X.; Rizzolio, M.; Breitenbucher, J. G.; McClure, K.; Shankley, N. P. *J. Pharmacol. Exp. Ther.* **2007**, in press, doi:10.1124/jpet.107.124578.
13. Biological CCK_2 binding data are contained in supplementary material.