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Synthesis and solid-phase purification of anthranilic sulfonamides as CCK-2 ligands

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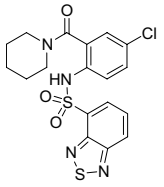
Abstract—A novel strategy for the synthesis of cholecystokinin-2 receptor ligands was developed. The route employs a solution-phase synthesis of a series of anthranilic sulfonamides followed by a resin capture purification strategy to produce multi-milligram quantities of compounds for bioassay. The synthesis was used to produce >100 compounds containing various functional groups, highlighting the general applicability of this strategy and to address specific metabolism issues in our CCK-2 program.

The cholecystokinin-2 receptor (CCK-2) remains an important target for the potential treatment of gastrointestinal disorders such as acid reflux, gastroesphogeal reflux disease, peptic ulcers[1] as well as GI adenocarcinoma.[2] In the 20 years since the disclosure of CCK-2,[3] no compounds targeting this receptor have become approved pharmaceuticals owing primarily to the variable pharmacokinetics and poor physiochemical properties of the clinical candidates.[3,4] Recently, we have re-examined CCK-2 as a target in our medicinal chemistry efforts in order to develop a small molecule antagonist with increased drug like properties.[5]

Testing of Johnson & Johnson compound library collection via high throughput screening identified a number of novel structural types showing good affinity for the CCK-2 receptor. Compound **1** exhibited good affinity ($pK_I = 6.4$) and selectivity over the related CCK-1 receptor ($pK_I < 5$). Pharmacokinetic (PK) analysis showed a moderate half-life (0.35 ± 0.03 h) and clearance (0.42 ± 0.01 L/kg/h) upon iv administration to the rat.



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To assess its moderate in-vivo half-life, an identification of the metabolites formed in the presence of human liver microsomes (HLM) was performed on **2** and analogs. Rapid oxidation of the benzothiadiazole and piperidine rings was found and hence could contribute significantly to the high in-vivo clearance (Fig. 1).[5]

To improve metabolic stability with respect to the benzothiadiazole moiety specifically,[6] a synthetic effort

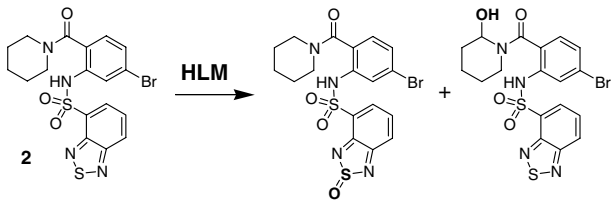

Figure 1. Metabolites of **2** formed in the presence of human liver microsomes.

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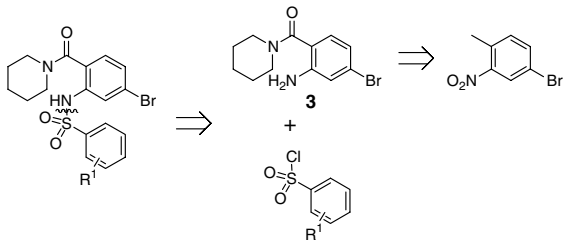
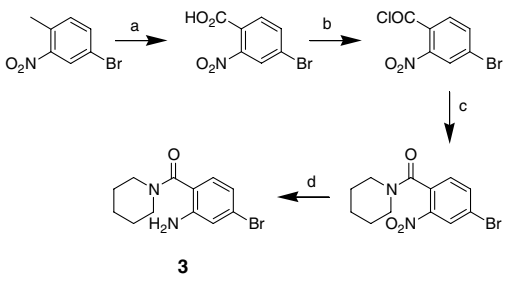


Figure 2. Retrosynthetic analysis of the anthranilic sulfonamides.



Scheme 1. Reagents and conditions: (a) $KMnO_4$, H_2O or *n*-Bu_4NMnO_4, pyridine, rt, 5–18 h; (b) $SOCl_2$, Δ; (c) piperidine, DCM, TEA; (d) $SnCl_2{\cdot}2H_2O$, EtOAc, CH_2Cl_2, rt.

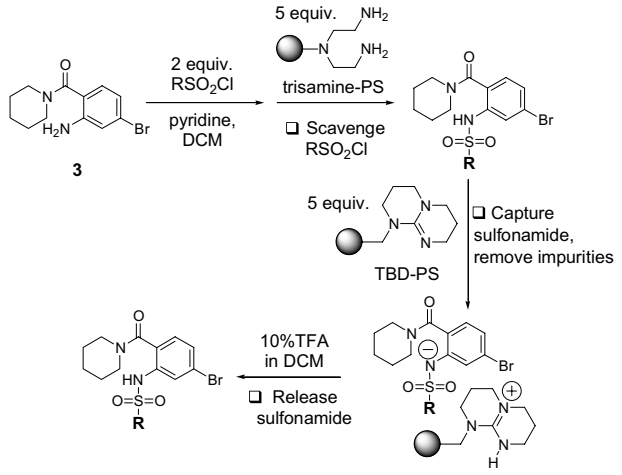
was undertaken to find a suitable replacement and provide compounds which maintain the same potency toward CCK-2, but offer improved resistance toward CYP 450 oxidation.

Retrosynthetic analysis of the anthranilic sulfonamides shows the sulfonamide linkage to be the logical disconnection in order to rapidly access arene-based replacements for the benzothiadiazole (Fig. 2).

The intermediate anthranilic amine (**3**) can be readily accessed in four steps from 4-bromo-2-nitro-toluene[5,7] (Scheme 1). The sulfonyl chloride components were obtained commercially and chosen to contain similar aromatic cores to the benzothiadiazole, but range in steric size, polar functionality, conformational preference, and overall polarity.

In order to streamline synthetic throughput, we decided to investigate and optimize a purification methodology for isolation of the proposed sulfonamide library. Automated liquid chromatographic purification systems of normal or reverse phase offer a viable, high throughput method for purification, however, these methods require a large volume of solvent and can be time consuming. Alternatively, purification schemes involving solid supported reagents or scavengers[8] offer the advantage of speed and ease of use, as reagents can simply be filtered from solution upon completion of reaction. Their usage, however, must be tailored to appropriately fit the chemistry at hand and are not suitable to all targets.

The anthranilic sulfonamides (such as **1** or **2**) bear an acidic sulfonamide NH proton, which can be deprotonated by a solid supported base and thus could in theory cause the anthranilic sulfonamide to be captured on a



Scheme 2. Synthetic and purification protocols for the production of anthranilic sulfonamides.

solid support through an ionic interaction. Components in the reaction mixture not containing acidic moieties could then be removed by filtration and the sulfonamide product later isolated by release from the solid support by treatment with an acidic solution. Similar 'catch and release' methods[9] using both ionic[10] and covalent[11] interactions have been used efficiently in library syntheses[12] of ethers,[13] amines,[14] sulfonates,[15] other moieties,[16] and in the alkylation of *N*-Boc sulfonamides,[17] however, to our knowledge, not for the purification of sulfonamides.[18]

To optimize the reaction and purification strategies (Scheme 2), we began by determining that addition of two equivalents of sulfonyl chloride in the presence of a mild base (pyridine) was sufficient to efficiently drive the reaction to completion in a reasonable time frame (approximately 1 h) and produce minimal amounts of bis-sulfonamide addition product. Minor formation of the latter was not expected to be an issue, however, as it would lack the requisite acidic NH sulfonamide proton, and hence be removed in our 'catch and release' purification strategy during washing.

To determine which solid supported base would be suitable for capturing the sulfonamide, we initially explored use of the trisamine-PS (tris-(2-aminoethyl)aminoethyl polystyrene resin) to both quench the excess sulfonyl chloride[19] and capture the desired final product. These efforts were only partly successful as the sulfonyl chloride was efficiently removed, however, capture of the final sulfonamide was inefficient. pK_a calculations show approximately two orders of magnitude difference between the acidic sulfonamide and basic trisamine-PS components,[20] which should have been suitable to our ends. Fortunately, the use of a more basic resin, TBD-PS (1,5,7-triazabicyclo[4.4.0]dec-5-ene polystyrene resin),[21] proved highly effective, giving quantitative recovery of the desired sulfonamide after capture and release under acidic conditions with trifluoroacetic acid in dichloromethane. Thus after these optimizations, we found that the overall transformation could be

completed in several hours and provide the desired sulfonamides in an average yield of 70% with an average purity of >95%.[22]

Using this methodology, we were able to produce >100 compounds for our CCK-2 program and rapidly investigate a variety of benzothiadiazole replacements possessing varied functionality. A representative cross section of the compounds is shown in Figure 3. Several different types of aryl and heteroaryl cores were produced including phenyls (**4a**–**g**), thiophenes (**4h**–**p**), furans (**4q**, **4r**), and other heterocycles (**4s**, **4t**). Many functional groups are represented which had the potential for acid instability such as esters (**4i**, **n**, **o**, **q**, **r**), amides (**4m**), and nitriles (**4e**), as well as basic amines in the core or periphery. Several moieties were found to be incompatible with this methodology, however, including compounds containing carboxylic acid, isoxazole, [1,2,4]-oxadiazole, and [1,2,4]-thiadiazole moieties, presumably due to either insolubility under reaction conditions or acid instability during the cleavage step.

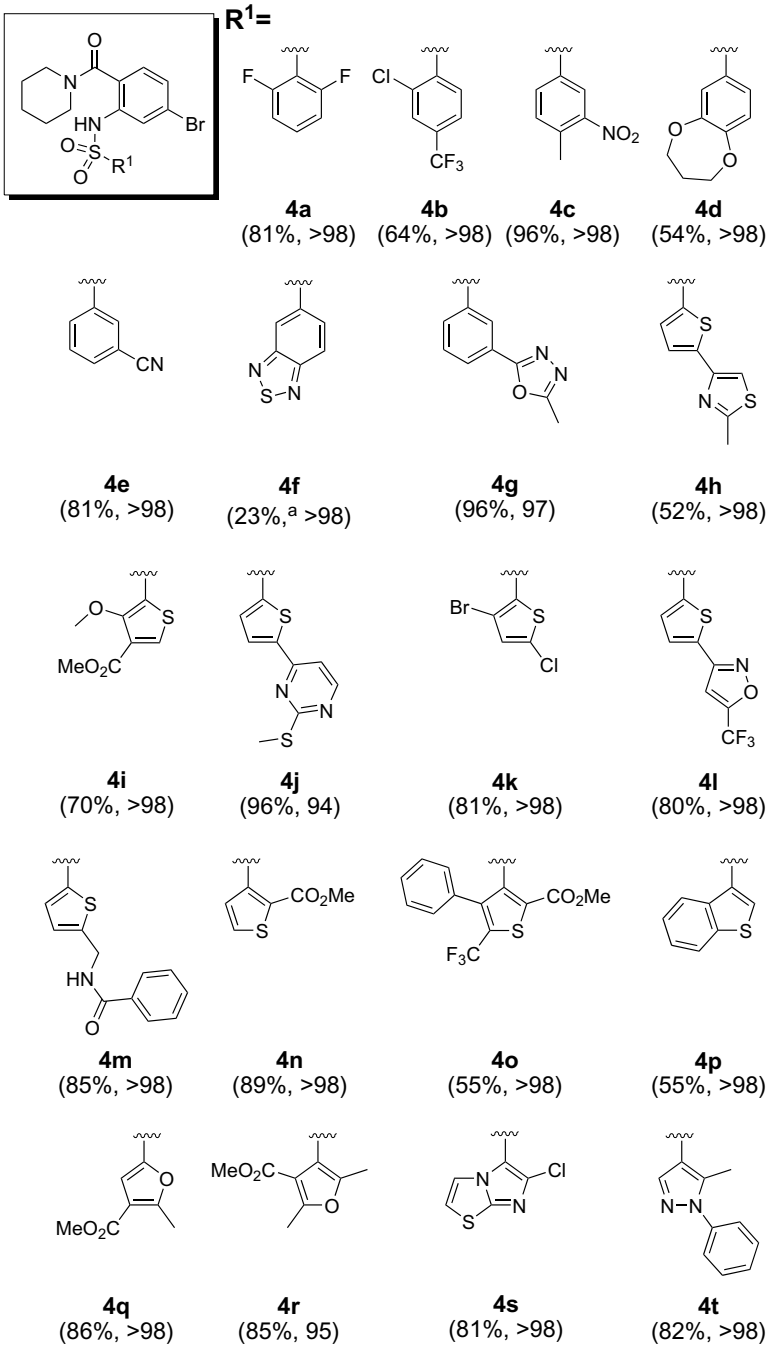


Figure 3. A cross-section of the compounds synthesized, showing functionality amenable to the solid-phase purification methodology described herein. Percent yield and purity are in parentheses below the compound number, respectively. [a]Purity of starting sulfonyl chloride was poor in this example contributing to low yield.

Table 1. Microsomal stability[a] and potency[b] for aryl sulfonamides **4a**–**t**

	HLM[a]	RLM[a]	pK_I[b]
2	0[c]	29	7.6
4a	77	78	6.3
4b	44	49	6.2
4c	3	25	5.7
4d	41	9	5.6
4e	7	100	5.3
4f	13	23	5.3
4g	0[c]	66	5.1
4h	5	47	5.4
4i	0[c]	0[c]	5.3
4j	63	39	5.5
4k	2	68	6.1
4l	89	87	5.3
4m	0[c]	0[c]	5.2
4n	3	37	5.8
4o	0[c]	0[c]	5.1
4p	23	19	5.5
4q	10	100	5.3
4r	0[c]	11	6.3
4s	7	100	5.8
4t	85	30	5.1

[a] Human liver microsome (HLM) and rat liver microsome (RLM) stabilities reported as percent of compound remaining after 30 min.
[b] Negative logarithm of the antagonist equilibrium dissociation constant calculated from the concentration required to displace 50% ^{125}I-CCK-8S (pIC_{50}) by the method of Cheng and Prussoff. All values are ±0.3 log units unless otherwise stated.
[c] Compound concentration was below detection limits.

The relative stability toward CYP 450 oxidation of the compounds was examined in the presence of human and rat liver microsomes (Table 1).[23] Several of the compounds showed good stability after 30 min. This was a vast improvement over compound **2** under the same conditions which showed rapid degradation.

Although we were able to achieve improved stabilities by this measure, receptor affinity values of the analogs (Table 1) were lower than for compound **2**. The best of the analogs from the library synthesis, **4a**, was over a full log unit lower relative to the direct benzothiadiazole analog **2**. Subsequently, in a second stage of this work, we undertook a chemical strategy to build structurally similar benzothiadiazole-like replacements to determine the requisite pharmacophore and modifiable regions of the system using X-ray crystallography, NMR, and computational studies and are subjects of future disclosures from our laboratories.[24]

In summary, we have developed an efficient and rapid synthetic methodology for the preparation of >100 anthranilic sulfonamides for our ongoing medicinal chemistry efforts. This methodology has produced a small library of compounds allowing us to refine our pharmacophore model for CCK-2 receptor binding of small molecule antagonists.

Acknowledgment

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Supplementary data

Characterization data for compounds **4a**–**t**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.087.

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21. pK_a calculations were made using ACD/pK_a DB, version 7.07, Advanced Chemistry Development, Inc., Toronto ON, Canada, www.acdlabs.com, 2006 and showed the pK_a of TBD-PS to be 13.5 based on the 1-(4-methyl-benzyl)-1,3,4,6,7,8-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine fragment of the resin.
22. Typical procedure for sulfonamide synthesis: To a 20 mL reaction vial were added amine **3** (50.0 mg, 0.18 mmol, 1 equiv), dichloromethane (10 mL) and pyridine (100 μL, 0.88 mmol, 5 equiv). Sulfonyl chloride (0.36 mmol, 2 equiv) was then added and contents agitated on a shaker plate for one hour or until reaction was complete by HPLC. Excess sulfonyl chloride was then removed by the addition of trisamine-PS resin (tris-(2-aminoethyl)aminoethyl polystyrene resin, 200 mg, 0.88 mmol, Aldrich, 4.4 mmol/g loading), shaking for 1 h, followed by filtration, and rinsing of the resin with dichloromethane. Purification of desired compound was carried out in a two-step 'catch and release' method by addition of TBD-PS resin (1,5,7-triazabicyclo[4.4.0]dec-5-ene polystyrene resin, 325 mg, 0.88 mmol, 5 equiv, Novabiochem, 2.7 mmol/g loading), shaking for 1 h, then collecting the resin containing the sequestered product via filtration and rinsing with dichloromethane. The desired product was released by shaking the resin with a 10% trifluoroacetic acid solution in dichloromethane (10 mL) for 30 min followed by filtration and removal of solvent under reduced pressure to yield the desired product as the trifluoroacetate salt. In several cases, the product obtained was an amber oil, but could easily be crystallized by the addition and subsequent trituration with diethyl ether.
23. Relative microsomal stability was assed by testing **2**, **4a**–**t** with the same batch of human and rat liver microsomes for thirty minutes under an identical protocol, followed by determination of the amount of parent remaining via LCMS analysis.
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