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Synthesis of spiro-2,6-dioxopiperazine and spiro-2,6-dioxopyrazine scaffolds using amino acids in a three-component reaction to generate potential Sigma-1 (σ_1) receptor selective ligands.

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Abstract

A library-friendly approach to generate new scaffolds is decisive for the development of molecular probes, drug like molecules and preclinical entities. Here, we present the design and synthesis of novel heterocycles with spiro-2,6-dioxopiperazine and spiro-2,6-pyrazine scaffolds through a three-component reaction using various amino acids, ketones, and isocyanides. Screening of select compounds over fifty CNS receptors including G-protein coupled receptors (GPCRs), ion channels, transporters, and enzymes through the NIMH psychoactive drug screening program indicated that a novel spiro-2,6-dioxopyrazine scaffold, **UVM147**, displays high binding affinity at sigma-1 (σ_1) receptor in the nanomolar range. In addition, molecular docking of **UVM147** at the human σ_1 receptor have shown that it resides in the same binding site that was occupied by the ligand 4-IBP used to obtain a crystal structure of the human sigma-1 (σ_1) receptor.

Graphical Abstract

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INTRODUCTION

The ability to generate diverse sets of molecular probes or drug-like molecules economically in a few steps facilitates the drug discovery process for the identification of molecular probes and novel preclinical entities.^{1,2} Isocyanide-based multicomponent reactions (IMCR) have proven efficient methods to produce a wide range of synthetic as well as natural product-based molecular probes and clinical drugs³⁻⁵ such as (Figure 1), xylocaine **1** (local anesthetic), praziquantel **2** (antiparasitic), boceprevir **3** (antiviral), 11-methoxy mitragynine pseudoindoxyl^{8,9} **4** and carfentanyl amide¹⁰ **5** (analgesics). Fundamental features of IMCR such as atom economy, single step, one-pot conversion, and the robust nature of the reaction enabling various functional moieties make IMCRs suitable for a range of synthetic chemistry fields.⁵ Methods and various strategies on advancing the IMCR on the emerging fields across chemistry, medicinal chemistry, and biology with innovative opportunities have been comprehensively reviewed by Domling^{11,12} and others.^{13,14}

Among the various IMCR approaches, the four-component Ugi multicomponent reaction (Scheme 1A) utilizing an isocyanide, aldehyde/ketone, amine, and carboxylic acids has been widely used for synthesizing numerous novel drugs and molecular probes.^{15,16} Three-component (Scheme 1B) variants of the Ugi reaction where one of the components such as carboxylic acid or amine does not participate in the reaction have also been explored equally to generate various scaffolds.^{17,18} Aiming to generate various biologically relevant synthetic scaffolds, we previously reported the synthesis of carfentanyl amides, via isocyanide-based four-component reaction to unveil **5** as an opioid analgesic.¹⁰ In addition, we reported the synthesis of benzoxazole heterocycles through three-component Ugi reaction (Scheme 1C), by reacting a bis-nucleophile such as 2-amino phenol with a ketone and an isocyanide, in which the isocyanide counterpart contributes only one carbon atom to the benzoxazole template through nitrilium trapping and displacement mechanism by the bis-nucleophile, amino phenol.¹⁹ However, the use of sterically hindered isocyanides such as 2,6-dimethylphenyl isocyanide (2,6 DMPC) with 2-amino phenol yield a benzoxazine heterocycle by incorporating the isocyanide-end group as a whole (Scheme 1D)¹⁹

Biologically active natural products and synthetic compounds containing 2,5-dioxopiperazine heterocycles²⁰⁻²² display a wide therapeutic potential including anticancer²³, antiviral²⁴, antifungal²⁵, and antibacterial activities.²⁶ They exhibit high affinities at GPCRs and various biological targets.²⁷⁻²⁹ However, there are limited studies on the closely related analogs spiro-2,6-dioxopiperazine and spiro-2,6-dioxopyrazine heterocycles³⁰⁻³³, thus, their potential scope is yet to be revealed. The usage of amino acids in IMCRs has been reported to prepare a range of heterocyclic scaffolds.^{34,35} Here, as a

continuation of our isocyanide based three-component reaction, we design and synthesize new spiro-2,6-dioxopiperazine and spiro-2,6-dioxopyrazine scaffolds through an IMCR approach for exploring their biological relevance. We started with *N*-methyl piperidine moiety, a privileged functional group found on various drug-like molecules, aiming to generate spiro-2,6-dioxopiperazine heterocyclic scaffolds (Scheme 1E).

Studies have shown that the majority of sigma-1 (σ_1) receptor ligands are heterocyclic in nature.³⁶⁻³⁸ Sigma-1 receptor is a chaperone protein found throughout the periphery and central nervous system (CNS) that modulates downstream signaling events of GPCRs and various other cellular processes.^{37,39} The physiological and functional role of σ_1 receptor, is yet to be revealed with a full scope.⁴⁰ Many of the σ_1 receptor-targeting ligands possess a piperidine core with a lipophilic framework including haloperidol, BD1063, 4-IBP, and norbenzomorphan, to name a few.^{37,41,42} To expand the library of diverse sets of piperidinyl heterocycles, we present the syntheses of spiro-2,6-dioxopiperazine scaffolds with piperidinyl group by utilizing various amino acids, ketones, and isocyanides (Scheme 2–6). In addition, several cycloalkyl analogs with or without an ether linkage have been prepared. A select group of compounds have been screened over fifty different CNS receptors including GPCRs, ion channels, transporters and enzymes through the psychoactive drug screening program (PDSP) offered by the National Institute of Mental Health (NIMH). The PDSP binding data indicated that the unsaturated spiro-2,6-dioxopyrazine scaffold **UM147**, a novel compound, is a potent (K_i : 8 nM) σ_1 receptor selective ligand.

RESULT AND DISCUSSION

To generate a library of various 2,6-dioxopiperazine scaffolds through the IMCR approach, we started with phenylalanine as a bis-nucleophile, *N*-methyl piperidone (NMP) as a cyclic ketone and 2,6-DMPC with no additional chemical catalyst (Scheme 2) by following the procedures described in the general method I. At first, various solvents including MeOH, EtOH, *i*PrOH, 2,2,2-trifluoroethanol (TFE), acetonitrile, THF, and dioxane were examined. The protic solvent 2,2,2-trifluoroethanol having a slight acidity ($pK_a = 12.37$)¹⁹ furnished an optimum yield of the desired spiro-2,6-dioxopiperazine heterocycle (**6**, 68%). As anticipated, no product was observed upon using aprotic solvents such as acetonitrile, THF and dioxane due to poor solubility of amino acids. On the other hand, the use of methanol, ethanol or isopropanol as solvent significantly decreased the yield of the desired product. For example, the yield of **6** decreased to 9% upon using methanol as solvent, along with the formation of an acyclic ester adduct in low yield (**7**, 16%) thereby being four-component reaction through the addition of the solvent. The phenomena of acyclic ester formation via a nucleophilic addition of alcohol into the reaction intermediate in multicomponent reactions has been previously reported.⁴³ Mild heat is required for these three-component reactions when using a ketone instead of an aldehyde and in some cases the yields were found slightly improved with an addition of an acid as a catalyst.^{10,19} However, we did not observe any increase in the yield of spiro-2,6-dioxopyrazine **6** when using various catalysts at the catalytic amount (10 mol %) under the same conditions (Scheme 2). The yields of **6** from strong acid catalyzed reactions were found to be 60% with dry HCl in dioxane, 62% for Pyridine. HCl, 52% for conc. H_2SO_4 and 60% for TfOH. The use of mild Lewis acid like $ZnCl_2$ (60%) and $Zn(OTf)_2$ (58%). furnished similar yields of **6**. An organic base catalyst,

triethyl amine catalyzed reaction slightly improved the yield (64%) which is still marginally lower compared to the yield of **6** with no catalyst. Thus we explored the scope of amino acid based three-component reaction towards the synthesis of spiro-2,6-dioxopyrazines catalyst free.^{10,19} Therefore, 60 °C was chosen as an ideal reaction temperature except in the case of spiro-2,6-dioxopyrazine analog formation described in Scheme 6 in which increased temperature improved the yields to some extent.

Aliphatic, aromatic or heteroaryl functionalities have been incorporated to the spiro-2,6-dioxopiperazine core in moderate to decent (**8–17**; 55–86%) yields (Scheme 3), by varying different types of amino acids while keeping the ketone and the isocyanide common. These amino acids include glycine, alanine, valine, leucine, isoleucine, proline, and methionine in the aliphatic series and tyrosine, histidine and tryptophan in the heteroaryl series.

No protection of the phenolic group of tyrosine or the heteroaryl moiety of histidine or tryptophan was required to furnish **15** (76%), **16** (86%), and **17** (74%), which can be advantageous for further functionalization following IMCRs.

On the other hand, as expected, no desired product was obtained upon using amino acids such as aspartic acid, glutamic acid, or arginine because of the additional reactive side chain. However, the use of 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) protected arginine gave the desired product but with low yield (**18**, 17%). These results indicate that reactive side chain moieties such as acid, amine and guanidinium groups require a protecting group to be incorporated into the 2,6-dioxopiperazine scaffolds. It is noteworthy that serine, cysteine and threonine possessing a beta (β)-heteroatom (O, S) furnished novel unsaturated analogs due to the elimination/dehydration followed by rearrangement of the double bonds spontaneously after IMCR as indicated in Scheme 6 which is described later separately.

Studies have shown that the presence of any spiro-center, another pharmacologically privileged moiety, displays unique tridimensional (3D) properties.⁴⁴⁻⁴⁶ The robustness of the amino acid-based three-component reaction to incorporate a range of ketones can be useful to access exceptionally diverse sets of spiro-2,6-dioxopiperazines. To expand the scope of generating spiro-cyclic compounds through IMCR, the 2,6-dioxopiperazine scaffold was diversified by varying different acyclic, cyclic, or heterocyclic ketones (Scheme 4). Unlike amino acid variation, the nature of the ketone revealed a noticeable effect on reaction progress and, subsequently, to the overall isolated yields, which is indicated in the scheme 4. For example, simply the replacement of *N*-methyl 4-piperidone with acetone or 2-butanone did not produce desired product, which is rather unanticipated. As described in the general protocol II, one equivalent of triethylamine was introduced to the reaction mixture to mimic the basicity of *N*-methyl-4-piperidone. The desired product **19** and **20** were observed by using mass spectrometry and were isolated in 43% and 29% yields, respectively. The low yield of **20** could be attributed to an increase in bulkiness of 2-butanone compared to that of **19**.

Similarly, the use of triethylamine increased the yields of several cyclic or heterocyclic ketone-derived products namely **21** (25%), **22** (37%), and **23** (58%), whereas only trace amount of the products was observed by mass spectrometry in the absence of triethylamine.

In contrast, the use of amino acid histidine along with same pair of cyclic ketone and isocyanide produced the desired product **24** (53%) and **25** (54%) without using triethylamine as a base. These yields obtained from using histidine are comparable to that of **21–23**, thereby suggesting that the basicity of histidine favors the product formation. It is important to note that no product was observed on using catalytic (0.1 equivalent) amount of triflic acid. Besides, *N*-allyl, *N*-benzyl or *N*-hexyl piperidone have been reacted with aryl amino acids histidine or tyrosine, by keeping the isocyanide counterpart common, to generate a few *N*-substituted piperidinyl spiro-2,6-dioxopiperazine scaffolds such as **26** (56%), **27** (91%), **28** (58%), and **29** (59%) with a range in isolated yields.

The isocyanide-end nitrogen atom occupies a key position (*N*1) in the 2,6-dioxopiperazine heterocyclic core. As shown above (Scheme 2–6), sterically demanding aryl isocyanide 2,6-DMPC can be an ideal isocyanide to deliver modest to excellent yields. Here, we report some examples to examine the possibility of diversification of 2,6-dioxopiperazine scaffolds through the isocyanide end by varying several aliphatic, benzyl and aryl isocyanides (Scheme 5). Benzyl group was introduced to the spiro-2,6-dioxopiperazine scaffolds (**30**, 75%) by using benzyl isocyanide. The use of cyclic or acyclic isocyanide produced 2,6-dioxopiperazine analogs (**31–34**; 49–71%) with moderate yields. A trend of increase in yields upon using cyclohexyl, cyclopentyl or cyclopropyl isocyanides suggested that a decrease in ring size of isocyanide end favors the yields of the desired product.

In addition, we showed that an ester moiety, an easily modifiable group after the synthesis of spiro-2,6-dioxopiperazine core, could be incorporated to 2,6-dioxopiperazine scaffolds such as **34** and **36** by using a desired isocyanide. As described in the general protocol II, addition of one equivalent triethylamine was required to furnish **36** (24%) by using cyclopentanone as a ketone resulted in low yield. Nevertheless, the yield is comparable to that of **21** (Scheme 4). An unanticipated low yield of **35** was found, when a methoxy aryl group was incorporated by using 4-methoxy isocyanide. Increase in temperature (up to 90 °C) and duration of the reaction (up to 48 hours) did not improve the yield. It is important to note that *t*-butyl isocyanide did not produce 2,6-dioxopiperazine based on mass spectrometry result of the crude reaction mixture. Instead, it gave an acyclic 2,2,2-trifluoroethyl ester adducts **37** and **38** with low yields as shown in Scheme 5.

Amino acids having a beta-hydroxy or thiol groups such as serine, threonine and cysteine displayed spontaneous elimination followed by rearrangement after the IMCR to produce new scaffolds. For instance, when serine was reacted with *N*-methyl-4-piperidone and 2,6-DMPC in TFE at 60 °C for 24 h, the desired product **39**, upon silica gel column chromatography, was obtained with a low yield of 35%. No yield improvement was found by increasing the reaction time alone. However, upon heating the reaction mixture at 72 °C for the same duration, mass spectrometry of the crude reaction mixture showed a new, unexpected product (LRMS; a major peak at 314.20), a loss of 18 amu compared to that of spiro-2,6-dioxopiperazine **39**, with a minor peak corresponding to **39**. Silica gel chromatography purification of the reaction mixture furnished the new product methyl spiro-2,6-dioxopyrazine **UVM147** with a 61% yield.

The novel methyl spiro-2,6-dioxopyrazine **UVM147** indicated a loss of alpha proton signal (no peak at 3.9 ppm) and an appearance of a methyl peak at 2.41 ppm in proton NMR compared to that of **39**. Also, ^{13}C Carbon NMR indicated an upfield shift of the amino acid-endo oxo-carbon signal from 170.0 ppm to 156.7 ppm because of a change at alpha carbon going from saturated (**39**) to unsaturated **UVM147** configuration. These preliminary data of mass spectrometry and NMRs were supportive initially to predict the structure of **41** that could potentially be formed (Scheme 6) upon the dehydration followed by rearrangement of **39**. Further, high resolution mass spectrometry results and X-ray crystallographic data of **UVM147** were analyzed to elucidate the exact structure of methyl spiro-2,6-dioxopyrazine **UVM147** (Figure 2). A few attempts were made to optimize the yield of the **UVM147** by either increasing reaction temperature, duration or both. An optimum yield (73%) of methyl spiro-2,6-dioxopyrazine **UVM147** was obtained upon conducting the reaction at 100 °C for 27 h, according to the general protocol III.

Interestingly, following the same protocol used for serine, cysteine furnished the same compound **UVM147** as the major product in a reduced yield of 7% at lower temperature (60 °C) with no indication of formation of thiol analog **40** based on mass spectrometry data of the crude reaction mixture. This result also suggests that the beta-thiol (SH) group can be eliminated upon cyclization to furnish the same product **UVM147** as seen in serine amino acid above but in a low yield of 7%. The yield was increased to 21% when the reaction mixture was carried out at 72 °C for 27 h and no further increase in yield was found upon increasing the heat of the reaction up to 100 °C for 27 h.

Similarly, threonine representing a methyl substituted serine furnished ethyl spiro-2,6-dioxopyrazine analog by showing similar kind of elimination of beta-hydroxy group followed by its rearrangement to release the final product **41**. Since the beta-hydroxy group elimination followed by rearrangement leads an unsaturation position at alpha (α) position, the use of amino acid such as alpha-methyl-serine could prevent the elimination. To test the hypothesis, a high-resolution mass spectrometry (HRMS) analysis of a test reaction using a mixture of alpha-methyl-DL-serine, by using the serine and threonine condition, showed a major peak of mass corresponding to that of alpha-methyl spiro-2,6-dioxopiperazine product (HRMS calculated for $\text{C}_{19}\text{H}_{28}\text{N}_3\text{O}_3$ ($\text{M}+\text{H}^+$); 346.2131; found 346.2123) with no sign of spiro-2,6-dioxopyrazine formation. No further analysis or purification of crude reaction mixture was pursued to separate the mixture of each isomer. Additionally, homoserine was used as a higher homologue of serine with an additional methylene unit in the side chain aiming to avoid the beta-hydroxy elimination effect at the similar reaction condition. Instead, the major product lactone **42** was obtained with 41% yield, conceivably through a rearrangement upon the lactonization of spiro-2,6-dioxopiperazine core as an intermediate by the side chain hydroxy group of homoserine, as a four-component adduct. More importantly, as anticipated, no product formation because of dehydration/elimination was observed on the mass spectrometry results of the crude reaction mixture.

With these all data on hand, we can comprehend that amino acids having only beta-hydroxy (or thiol) group furnish the spiro-2,6-dioxopyrazine analogs. The spiro-2,6-dioxopiperazine intermediate **39** upon beta-OH elimination conceivably through enolate of aldol **47** resulting in exocyclic unsaturated intermediate **48** followed by its spontaneous rearrangement could

furnish **UVM147** as the final product (Scheme 7). However, no further efforts were pursued at this stage for identification or characterization of any intermediates proposed **47–48** in the mechanism (Scheme 7).

Finally, we synthesized a few close analogs of spiro-2,6-dioxopyrazine **UVM147** to find out its potential scope on synthetic diversification through IMCR. Heterocyclic and cycloalkyl analogs of **UVM147** such as tetrahydro-4H-pyranyl spiro-2,6-dioxopyrazine **43** and cyclohexyl spiro-2,6-dioxopyrazine **44** were prepared in modest yields of 65% and 67% respectively by heating the equimolar mixture of reactants at 100 °C for 24 h in the presence of one equivalent of triethyl amine (Scheme 6). In addition, spiro-2,6-dioxopyrazine analogs **45** and **46**, having either a sterically less demanding benzyl group or a small aliphatic cyclopropyl moiety, were prepared in 30% and 24% yields. Key structural features of spiro-2,6-dioxopyrazines (**41**, **43–46**) includes an ethyl substitution at alpha-carbon in **41**, a tetrahydro-4H-pyranyl group in **43**, a cyclohexyl ring in **44**, and a *N*-benzyl or *N*-cyclopropyl group in **45** or **46** respectively.

Meanwhile, select compounds - i) having a methylene unit with no substitution at alpha carbon **8**; ii) having a substitution of pharmacologically privileged indole moiety **17**; iii) having a terminal hydroxy group to facilitate hydrogen bonding **39**; and iv) having unsaturated novel spiro-2,6-dioxopyrazine core **UVM147** were submitted to screen potential biological targets through NIMH-Psychoactive Drug Screening Program (PDSP). With the initial screening of over fifty CNS receptors including GPCRs, ion channels, transporters and enzymes, it was stimulating to find out that the serine analogs **39** and **UVM147** displayed binding affinities for the sigma-1 receptor. **UVM147** (K_i , 8.6 nM) is a potent sigma-1 ligand (Table 1), while the serine analog **39** showed a moderate but selective binding (K_i , 58 nM) to sigma-1 over sigma-2 and other several CNS targets. More importantly, **UVM147** is also selective for sigma-1, displaying weak or no binding affinity at several other crucial biological targets, thereby indicating a potential advantage of the spiro-2,6-dioxopyrazine scaffold.

Previously it was reported that a heterocyclic ligands 1,2,3,6- tetrahydro-5-[3-(4-methylphenyl)-5-isoxazolyl]-1-propylpyridine (PD144418)⁴⁷ and *N*-(*N*-benzylpiperidin-4-yl)- 4-iodobenzamide (4-IBP)⁴⁸ were used successfully to crystallize the human sigma 1 receptor.⁴² These two ligands PD144418 and 4-IBP, having a tetrahydro-4H-pyridinyl or a piperidinyl functionality as a biologically privileged moiety, bind the sigma-1 receptor in comparable fashion.⁴² Since our novel methyl spiro-2,6-dioxopyrazine **UVM147** has shown a binding affinity at the nanomolar level, we wanted to explore its potential binding poses through molecular docking at the sigma-1 receptor in comparison to that of the 4-IBP (Figure 3 A–C). First, as a reference, our docking results of 4-IBP with the best scored predicted pose at sigma-1 receptor (PDBID:H5K2) have shown that the tertiary nitrogen in 4-IBP forms a salt bridge with Glu172 (Figure 3B) and displays extensive hydrophobic contacts between aryl ring with other residues Leu 105 and Leu 95. These docking prediction results (Figure 3B) are in agreement with that of the 4-IBP ligand residing at the true position in the co-crystal structure (Figure 3C) of sigma-1 receptor.⁴²

Next, the methyl spiro-2,6-dioxopyrazine **UVM147** was docked into the binding site of the sigma-1 receptor model. Our docking results showed that **UVM147** occupies the similar binding site as 4-IBP at the sigma-1 receptor (Figure 3A) by displaying matching contacts between piperidinyl nitrogen and Glu172 through a salt bridge. In addition, the hydrophobic contacts observed includes i) between the 3-methyl group and Met93 and ii) among methyl groups of aryl ring with Leu105 and Leu95.

Taken together, by considering the synthetic scope, preliminary binding data and the analysis of the docking results of **UM147**, we can propose that these novel spiro-2,6-dioxopyrazine analogs could be explored as the potential templates to generate sigma-1 (σ 1) selective ligands through three-component reaction fashion. The next series of analogs will utilize structure-based design to enhance potency as well as selectivity using **UVM147** as well as **39** as initial leads.⁴⁹ Studies have shown that sigma-1 (σ 1) receptor is capable in modulating a variety of cellular processes including depression⁵⁰, drug addiction⁵¹, neurodegeneration⁵², and neuropathic pain.⁵³ Given the importance on various roles of sigma-1 (σ 1) receptor in biology, these tools and methods enabling to generate diverse sets of analogs through a rational design in an economic fashion will be useful to conduct a detail SAR on sigma 1 receptor in the future.

CONCLUSION

As the continuation of generating a library of diverse entities, we used various amino acids as bis-nucleophiles, aliphatic as well as aromatic isocyanides and cyclic ketones to the synthesis of a small library of new spiro-2,6-dioxopiperazine and spiro-2,6-dioxopyrazine heterocycles as novel scaffolds potentially targeting sigma-1 (σ 1) receptor. This simple approach can utilize commercially available materials such as amino acids, ketones, and isocyanides to produce distinct class of heterocycles having potential biological implication at the sigma-1 receptor. Essential features including atom economy, single step conversion as a one-pot reaction, use of non-aqueous protic solvent trifluoroethanol and a mild heating condition could enable this IMCR method accessible to various levels of laboratories.

EXPERIMENTAL

Chemistry:

Chemicals were purchased from Sigma-Aldrich Chemicals, Fisher scientific, Alfa Aesar, Chem-Impex international nt., Bachem and were used without further purification. Reaction mixtures were purified by silica gel flash chromatography on E. Merck 230–400 mesh silica gel 60 using a Teledyne ISCO CombiFlash R_f instrument with UV detection at 280 nm and 254 nm. RediSep R_f silica gel normal phase columns were used. The yields reported are isolated yields. NMR spectra were recorded on Bruker Avance III 600 with DCH CryoProbe instruments. Chemical shifts are reported in parts per million (ppm) relative to residual solvent peaks at the nearest 0.01 ppm for proton and 0.01 ppm for carbon (CDCl₃ ¹H: 7.26, ¹³C: 77.10). Peak multiplicity is reported as the NMR spectra were processed with MestReNova software, such as s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet. Coupling constant (*J*) values are expressed in Hz. Mass spectra were obtained at the MSKCC Analytical Core Facility using The Waters Acuity SQD LC MS by electrospray

(ESI) ionization. High resolution mass spectra were obtained on a Waters Acuity Premiere XE TOF LC-MS by electrospray ionization. Accurate masses (HRMS) data are reported for the molecular ion $[M+H]^+$.

Procedure:

All the reactions were conducted as the equimolar concentration of all the reactants with either 0.44 mmol or 0.18 mmol scale as indicated in the schemes 3–6 by following any one of the general methods (I–III) below. **General method I: Three-component reaction using N-substituted-4-piperidones with various amino acids;** Ketone (1 eq.) was dissolved in trifluoroethanol (2.0 mL) at room temperature. Amino acid (1 eq.) and isocyanide (1 eq.) were added into the solution. The mixture was heated at 60 °C and the progress of reaction was monitored by mass spectrometry. After 24 hours, the reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. Then, the mixture was redissolved in DCM (0.5 mL) and was directly purified by using silica gel column chromatography with 1-10 % MeOH in DCM. The product fraction, based on mass spectrometry, was collected, solvent was removed, and the content was dried under reduced pressure to get the desired products.

General method II: Three-component reaction using acyclic and cyclic ketones devoid of tertiary amine.

Ketone (1 eq.) was dissolved in trifluoroethanol (2.0 mL) at room temperature. Amino acid (1 eq.) and isocyanide (1 eq.) were added into the solution. The, one equivalent of triethyl amine was added to the reaction and the mixture was heated at 60 °C. After 24 hours, the reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. Then, the mixture was redissolved in DCM (0.5 mL) and was directly purified by using silica gel column chromatography with 1-5 % MeOH in DCM. The product fraction, based on mass spectrometry, was collected, solvent was removed, and the content was dried under reduced pressure to get the desired products.

General method III: Three-component reaction using Serine and threonine analogs.

Ketone (1 eq.) was dissolved in trifluoroethanol (2.0 mL) at room temperature. Amino acid (1 eq.) and isocyanide (1 eq.) were added into the solution. The, one equivalent of triethyl amine was added to the reaction and the mixture was heated at 100 °C. After 24 hours, the reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. Then, the mixture was redissolved in DCM (0.5 mL) and was directly purified by using silica gel column chromatography with 1-5 % MeOH in DCM. The product fraction, based on mass spectrometry, was collected, solvent was removed, and the content was dried under reduced pressure to get the desired products.

Crystal Structure Determination

A colorless plate-like crystal with the size of $0.09 \times 0.32 \times 0.38 \text{ mm}^3$ was selected for geometry and intensity data collection with a Bruker SMART APEXII CCD area detector on a D8 goniometer at 250 K. The temperature during the data collection was controlled with an Oxford Cryosystems Series 700+ instrument. Preliminary lattice parameters and

orientation matrices were obtained from three sets of frames. Data were collected using graphite-monochromated and 0.5 mm-MonoCap-collimated MoK α radiation ($\lambda = 0.71073$ Å) with the ω scan method.⁵⁴ Data were processed with the INTEGRATE program of the APEX2 software^[1] for reduction and cell refinement. Multi-scan absorption corrections were applied by using the SCALE program for the area detector. The structure was solved by the direct method and refined on F² (SHELXTL)⁵⁵. Non-hydrogen atoms were refined with anisotropic displacement parameters, and hydrogen atoms on carbons were placed in idealized positions (C-H = 0.94-0.98 Å) and included as riding with $U_{\text{iso}}(\text{H}) = 1.2 U_{\text{eq}}(\text{non-H})$ and hydrogen atoms on nitrogen atoms were refined isotropically with a restrained N-H distance of 0.86 Å

Molecular docking

The UVM147 and 4-IBP ligands were prepared using LigPrep (Schrödinger) with up to six ring conformations and 32 stereoisomers and force field 16. The grid for the sigma-1 receptor (PDBID:H5K2) was performed using default parameters and a 20 Å cube bounding box. Standard precisions docking was performed for both ligands using Glide (Schrödinger) with a max time of 300s.^{56,57} The energy minimized poses for each ligand were visualized using PyMol.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

DCM	dichloromethane
MeOH	methanol
EtOH	ethanol
iPrOH	isopropanol
AcN	Acetonitrile
THF	tetrahydrofuran

NMP	<i>N</i> -methyl-4-piperidone
2,6-DMPC	2,6-dimethylphenyl isocyanide
TFE	2,2,2-trifluoroethanol
IMCR	Isocyanide-based multicomponent reaction
4-IBP	<i>N</i> -(<i>N</i> -benzylpiperidin-4-yl)-4-iodobenzamide
Met	Methionine
Leu	Leucine
Glu	Glutamic acid
nM	nanomolar
amu	atomic mass unit
int	intermediate
pdt	product
CNS	Central nervous system
GPCRs	G-protein coupled receptors
NMDA	<i>N</i> -methyl-D-aspartate receptor
MOR	<i>mu</i> opioid receptors
KOR	<i>kappa</i> opioid receptors
GP	Guinea pig
PC₁₂	rat pheochromocytoma-12

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Highlights

- Novel spiro-2,6-dioxopiperazine and spiro-2,6-pyrazine heterocycles were synthesized.
- The structure of methyl spiro-2,6-dioxopyrazine **UVM147** was determined by X-ray crystallographic data analysis.
- The methyl spiro-2,6-pyrazine **UVM147** (K_i , 8.6 nM) is a potent ligand at sigma-1 (σ_1) receptor.

Figure 1.
Biologically relevant compounds derived from isocyanide based multicomponent reaction (IMCR) approach

Figure 2:
Molecular structure of methyl spiro-2,6-dioxypyrazine **UVM147** based on x-ray crystallographic analysis.

Figure 3: Docking pose of ligands at sigma-1 (σ_1) receptor; Methyl spiro-2,6-dioxopyrazine **UVM147** (A); the ligand 4-IBP with the best scored prediction pose (B); and the 4-IBP ligand residing at the true position in the co-crystal structure (C)⁴².

A. Four-component Ugi reaction **B. Three-component Ugi reaction** **C. Three-component Ugi reaction with amino phenol** **D. Three-component Ugi reaction with amino phenol using sterically hindered isocyanide** **E. Three-component Ugi reaction with amino acid**

Moities: R^1, R^2 = aliphatic, aromatic; R^3, R^4 = aliphatic (acyclic/cyclic); R^5 = aliphatic, aromatic;

* Sterically hindered isocyanide such as 2,6-dimethylphenyl isocyanide (2,6-DMPC)

Scheme 1.

Isocyanide based three and four-component reactions

Scheme 2:

Three-component reaction producing spiro-2,6-dioxopiperazine (at 0.4 mMol)

Scheme 3:

Variation of amino acid end in three-component reaction (at 0.4 mMol)

Scheme 4:

Variation of ketone end in three-component reaction (at 0.4 mMol)

Scheme 5:

Variation of isocyanide end in three-component reaction (at 0.18 mMol)

Scheme 6:

Beta-hydroxy moiety leads to alkyl spiro-2,6-dioxypyrazine (at 0.4 mMol)

Scheme 7.

Proposed mechanism of dehydration followed by rearrangement leading spiro-2,6-dioxypyrazine

Table 1.

Binding data (Ki, nM) of select compounds through PDSP Screening

8	17	39	UVM147							
Cmpd.	5-HT2B	5-HT7	Alpha2A	D4	DAT	H2	KOR	MOR	Sigma-1 GP	Sigma 2
8	742	–	–	–	–	–	–	10000	–	–
17	840	–	–	–	–	546	–	993	–	–
39	2051	–	–	–	–	–	–	1634	58	–
UVM147	231	2765	3364	1283	2562	823	1707	–	8.6	696

* Serotonin receptor (5-HT2B, 5-HT7), alpha 2 Adrenergic receptor (Alpha2A), Dopamine receptor (D4), Dopamine Transporter (DAT), Histamine 2 receptor (H2), opioids receptors (KOR, MOR), and Sigma receptors (Sigma-1 GP, Sigma-2 PC12).

Binding affinities reported were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP).Details of the methods and radioligands used for the binding assays are available on the NIMH PDSP website at <https://pdspdb.unc.edu/pdspWeb/content/PDSP%20Protocols%20II%202013-03-28.pdf>. Blanks represent binding affinity of Ki > 10 μM.