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Screening of 5-HT$_{1A}$ Receptor Antagonists using Molecularly Imprinted Polymers

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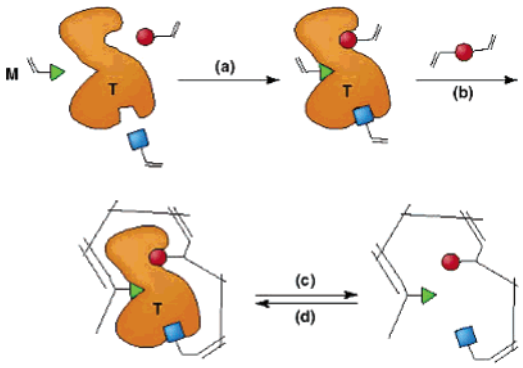
Abstract: Molecular imprinting produces network polymers with recognition sites for imprint molecules. The high binding affinity and selectivity in conjunction with the polymers' physical robustness positions molecular imprinted polymers (MIPs) as candidates for use as preliminary screens in drug discovery. As such, MIPs can serve as crude mimics of native receptors. In an effort to evaluate the relationship between MIPs and native receptors, imprinted polymers for WAY-100635, an antagonist of the serotonin (5-HT) receptor subtype 5-HT$_{1A}$ were prepared. The resulting MIP **P(WAY)** was evaluated as an affinity matrix in the screening of serotonin receptor antagonists with known affinities for the native receptor. Rough correlations in affinity between the synthetic **P(WAY)** and native receptor 5-HT$_{1A}$ were found. These findings provide some support for the analogy between MIPs and native receptors and their possible use as surrogates.

Introduction

Molecular imprinting is a general method for preparing synthetic receptors. Molecularly imprinted polymers (MIPs) can have substrate affinities comparable to those of antibodies (Scheme 1)[1−4] and have been evaluated for a wide variety of applications including chromatographic stationary phases,[5,6] solid-phase extraction materials,[7] catalysts,[8,9] and as chemical and biomimetic sensors.[10−13]

The high binding affinity in conjunction with the physical robustness of MIPs also positions them as candidates for use as screens in drug discovery.[14−21] Indeed, it has often been stated that MIPs created from imprint molecules with high affinity for biological receptors may serve as a crude mimic of the native receptor. Implicit in the use of MIPs as a screen for biologically active compounds are parallels in ligand binding between the MIP and the native receptor. Is there justification for this assumption? What evidence is there that the binding site of a MIP made from a high affinity ligand will resemble the native binding site? Protein receptors have domains created by

Scheme 1. Schematic Diagram of the Molecular Imprinting Process



[a] Conditions: (a) Formation of prepolymerization complex between template T and functional monomers M; (b) copolymerization with an excess of cross-linking monomer; (c) extraction of template from imprinted binding sites using porogen; (d) rebinding of the template to imprinted binding sites.

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(1) Yan, M., Ramstrom, O., Eds.; *Molecularly Imprinted Materials: Science and Technology*; Marcel Dekker: New York, 2005.
(2) Komiyama, M.; Takeuchi, T.; Mukawa, T.; Asanuma, H. *Molecular Imprinting: From Fundamentals to Applications*; Wiley-VCH: Weinheim, 2003.
(3) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166−73.
(4) Alexander, C.; Andersson, H. S.; Andersson, L. I.; Ansell, R. J.; Kirsch, N.; Nicholls, I. A.; O'Mahony, J.; Whitcombe, M. J. *J. Mol. Recognit.* **2006**, *19*, 106−180.
(5) Kempe, M.; Mosbach, K. *J. Chromatogr., A* **1995**, *694*, 3−13.
(6) Mayes, A. G.; Mosbach, K. *Trends Anal. Chem.* **1997**, *16*, 321−332.
(7) Andersson, L. I. *Bioseparation* **2002**, *10*, 353−364.
(8) Ramstrom, O.; Mosbach, K. *Curr. Opin. Chem. Biol.* **1999**, *3*, 759−764.
(9) Wulff, G. *Chem. Rev.* **2002**, *102*, 1−27.
(10) Ansell, R. J.; Kriz, D.; Mosbach, K. *Curr. Opin. Biotechnol.* **1996**, *7*, 89−94.
(11) Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468−475.
(12) Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495−2504.
(13) Kriz, D.; Ramstrom, O.; Mosbach, K. *Anal. Chem.* **1997**, *69*, 345A-349A.
(14) Deng, Q.; Lun, Z.; Shao, H.; Yan, C.; Gao, R. *Anal. Bioanal. Chem.* **2005**, *382*, 51−58.
(15) Ye, L.; Haupt, K. *Anal. Bioanal. Chem.* **2004**, *378*, 1887−1897.
(16) Bouman, M. A. E.; Allender, C. J.; Brain, K. R.; Heard, C. M. In *Methodological Surveys in Bioanalysis of Drugs*; Reid, E., Hill, H. M., Wilson, I. D., Eds.; Royal Society of Chemistry, Information Services: Cambridge, UK, 1998; Vol. 25, pp 37−43.
(17) Ramstrom, O.; Ye, L.; Mosbach, K. *Anal. Commun.* **1998**, *35*, 9−11.
(18) Khasawneh, M. A.; Vallano, P. T.; Remcho, V. T. *J. Chromatogr.*, A **2001**, *922*, 87−97.
(19) Vallano, P. T.; Remcho, V. T. *J. Chromatogr., A* **2000**, *888*, 23−34.
(20) Xu, X.; Zhu, L.; Chen, L. *J. Chromatogr., B* **2004**, *804*, 61−69.
(21) Ye, L.; Yu, Y.; Mosbach, K. *Analyst* **2001**, *126*, 760−765.

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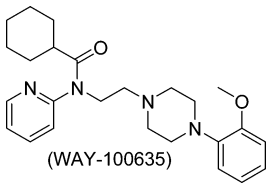


Figure 1. 5-HT_{1A} selective antagonist WAY-100635.

polypeptides. The binding domains are complementary in shape to the substrate and possess an array of strategically positioned functional groups that enter into numerous weak binding interactions of the substrate. Molecular imprinting creates domains in crosslinked network polymers that are complementary in shape to the template molecule. The imprinting process is also believed to position a limited range of complementary functional groups in three-dimensional space that provides for the recognition of analyte molecules.[22−24] Upon closer examination, however, the similarities wane. Protein receptors (i.e., antibodies) have been refined through eons of evolutionary fine-tuning. They employ a wide assortment of complementary functionality for recognition. Once perfected, biological receptors are easily replicated. MIPs on the other hand have binding sites produced under kinetic control. There is no simple method to correct or refine them. They are fabricated from organic monomers and a limited range of complementary functional groups so that there is no correspondence at the molecular level with native receptors. Furthermore, MIP binding sites are polyclonal.

Although the molecular composition of MIP binding sites may not mimic that of the native protein, they may however respond similarly to variations in ligand structure and thus prove useful for identifying candidates with similar affinities. To evaluate the correspondence between MIPs and native receptors, we describe the synthesis and evaluation of MIPs as an affinity assay for antagonists of the serotonin receptor subtype 5-HT_{1A}. We chose WAY-100635, an antagonist of the serotonin receptor subtype 5-HT_{1A} with nanomolar affinity, as our imprint molecule[25] (Figure 1).We then investigated the affinity of the WAY-100635 MIPs (**P(WAY))** for WAY-100635 and its analogues. The analogues of WAY-100635 have known affinities for the receptor and thus comparisons between P(WAY) and the native receptor could be made.

Serotonin is a neurotransmitter and with its receptors has been shown to be involved in several neurological and psychiatric diseases (e.g., anxiety, depression, feeding disorders, schizophrenia, Alzheimer's disease, and sexual disorders).[26] The mechanism of regulation by these receptors remains unclear. WAY-100635 was developed as the first potent, "silent" and selective 5HT_{1A} receptor antagonist.[25,26] This compound has an IC_{50} value of 2.2 nM at the rat hippocampal 5-HT_{1A} receptor and selectivity in binding of over 100-fold relative to binding at other 5-HT receptor subtypes and major neurotransmitter receptor, reuptake, and ion channel sites.

The 5-HT receptors are G protein-coupled receptors (GPCRs) consisting of a single polypeptide chain that is integrated into the plasma membrane of cells via seven transmembrane domains. The transmembrane and extracellular portion of the receptor form the ligand recognition domain, and the intracellular portion forms the coupling domain that interacts with guanine nucleotide binding proteins (G-proteins). The design of selective ligands for 5-HT receptors represents a considerable challenge. Far less is known about the structure of membrane-bound proteins than about that of soluble proteins, as X-ray crystal structures have proven difficult to obtain.[27] Not knowing the structure of the 5-HT receptor makes it difficult to design molecules that target specific protein domains.

5-HT_{1A} receptor mimics present an ideal opportunity for MIPs.[15−21] The 5-HT_{1A} receptor is an important medicinal target, and thus synthetic mimics could prove to be a useful tool. We chose WAY-100635 as the imprint molecule as it is one of the tightest binding with an IC_{50} of 1.35 nM.[28] The molecule is also highly selective with >100-fold selectivity for the 5-HT_{1A} site relative to selectivity for a range of other CNS receptors, the implication being that this will produce a MIP that most closely approximates the native receptor. To test this hypothesis we evaluated the MIP **P(WAY)** affinities for a range of WAY-100635 analogues and compared these to that for the native receptor (5-HT_{1A}).

Zhuang et al. have shown in a series of benzamido analogues of WAY-100635 that there is a limited tolerance for variations at the amino pyridine position in the native receptor.[29] As the hydrocarbon substituents at the para position become more sterically demanding, a decrease in affinity for the native receptors is observed. We set out to investigate if similar size constraints are found with the use of the MIP **P(WAY)** and if any correlation can be drawn between 5-HT_{1A} binding affinites and affinity for the MIP **P(WAY)**.

Results and Discussion

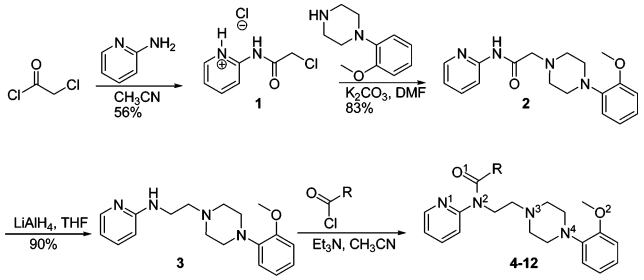
Affinity Ligand Synthesis. Structural analogues of WAY-100635 (**5**−**12**) of known nanomolar affinity to 5-HT_{1A} were prepared from piperazinyl **3**[29] (see Scheme 2).

MIP Synthesis. WAY-100635 (**4**) was used as the imprint molecule for preparation of cross-linked ethylene glycol dimethacrylate (EDGMA) polymers with methacrylic acid (MAA) as the functional monomer (Scheme 3). Solutions of template (1 mol %), MAA (11 mol %), EGDMA (87 mol %), and 2,2-dimethoxy-2-phenyl acetophenone (DMPA) (1 mol %) in chloroform were polymerized photochemically by UV irradiation (a Hanovia medium-pressure 500 W mercury arc lamp) for 24 h at 5 °C to yield polymers imprinted with WAY-100635 **P(WAY**). Control polymers containing the same molar ratio of monomers but incorporating a "generic" template, benzylamine, **P(BA)**, or without template **P(BL)** were also prepared. The resulting polymers were crushed and subjected to Soxhlet extraction with methanol. The extracts were evaporated to dryness and dissolved in methanol (10 mL). WAY-100635 was recovered in >89% (i.e., splitting yield) as determined by reverse-phase HPLC. Further release of WAY-100635 was not observed in subsequent washings.

Following removal of the template molecule, the polymers were then ground and sieved to a 38−125 μm particle size for

(22) Becker, J. J.; Gagne, M. R. *Acc. Chem. Res.* **2004**, *37*, 798−804.
(23) Conrad, P. G., II; Shea, K. J. In *Molecular Imprinted Materials: Science and Technology*; Mingdi, Y., Ramstrom, O., Eds.; Marcel Dekker: New York, 2005; pp 123−180.
(24) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1989**, *111*, 3442−3444.
(25) Forster, E. A.; Cliffe, I. A.; Bill, D. J.; Dover, G. M.; Jones, D.; Y. R.; Fletcher, A. *Eur. J. Pharmacol.* **1995**, *281*, 81−88.
(26) Cliffe, I. A.; Fletcher, A. *Current Drugs: Serotonin* **1993**, *1*, 99−124.
(27) Eisenberg, D. *Annu. Rev. Biochem.* **1984**, *53*, 593−623.
(28) Fletcher, A.; et al. *Behav. Brain Res.* **1996**, *73*, 337−353.
(29) Zhuang, Z.; Kung, M.; Chumpradit, S.; Mu, M.; Kung, H. F. *J. Med. Chem.* **1994**, *37*, 4572−4575.

Scheme 2. Synthesis of WAY-100635 and Analogues



R (Name)	R (Structure)	% Yield
Cyclohexyl, **4** (WAY-100635)		86%
Adamantyl, **5**		85%
4-Iodophenyl, **6**		88%
Phenyl, **7**		90%
4-Propylphenyl, **8**	C_3H_7	86%
4-Pentylphenyl, **9**	C_5H_{11}	86%
4-Heptylphenyl, **10**	C_7H_{15}	76%
4-Fluorophenyl, **11**	F	75%
2-Furoyl, **12**		70%

rebinding experiments and 25−38 μm particle size for chromatography experiments. For rebinding experiments, 20 mg of dry polymer was suspended in 1-mL stock solutions of WAY-100635 for 24 h, and the changes in concentration were obtained using reverse phase HPLC. For chromatography experiments, polymer particles were slurry packed in 10 cm × 0.46 cm i.d. stainless steel chromatography columns for evaluation by HPLC. Approximately 1 g of polymer was required to pack one column. Once packed, the columns were washed with the various eluents of choice until a stable baseline was obtained before analysis.

The resulting polymers have a high degree of chemical and thermal stability. They are mechanically robust and can endure high pressures as HPLC column packing materials without fracturing. Their physical characteristics from N_2 adsorption analysis are given in Table 1. The polymers all have low surface

Table 1. Physical Characteristics of Molecular Imprinted and Control Polymers

characteristics	P(WAY)	P(BA)	P(BL)
surface area (m^2/g)	5.1	1.1	5.9
pore volume (mL/g)	∼0	∼0	∼0

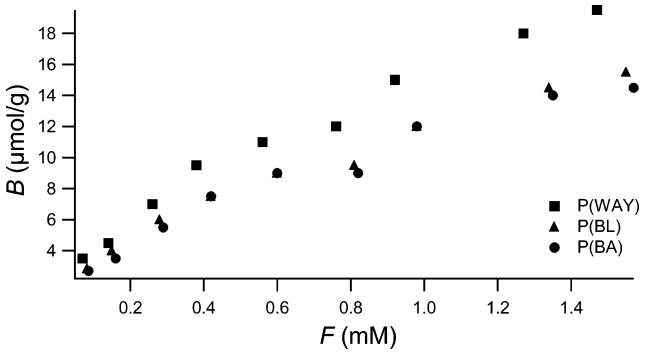


Figure 2. Binding isotherms of **P(WAY)**, **P(BA)**, and **P(BL)**.

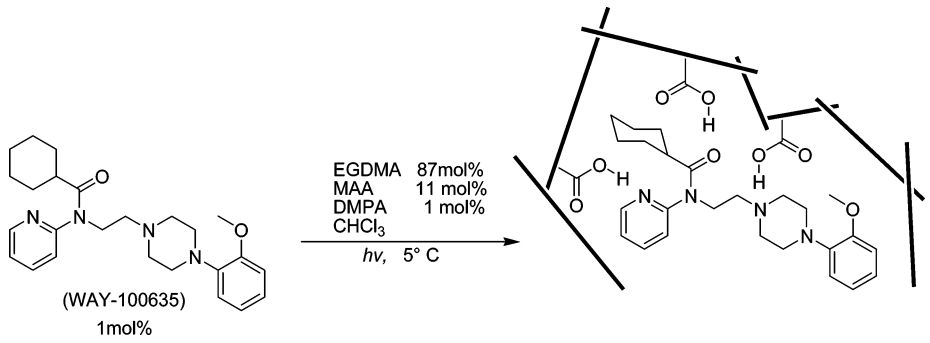
areas in line with N_2 adsorption analysis of previously made imprinted materials with $CHCl_3$ as porogen.[30] This also demonstrates that both imprinted and control materials are of similar nature. It should be noted, however, that the low surface areas and pore volumes determined in the dry state do not represent the porosities of these polymers in the presence of solvents.

Batch rebinding experiments were performed to evaluate the affinity of WAY-100635 for the imprinted and control polymers. Polymers were suspended in stock solutions of WAY-100635 in $CHCl_3$ for 24 h, and then the concentration of WAY-100635 was measured. The equilibrium concentration of free WAY-100635 is labeled as ***F***. The concentration of WAY-100635 bound to polymer is designated ***B*** and is computed by taking the difference between the total concentration of WAY-100635 (***T***) and ***F*** ($B = T - F$).

The binding isotherms of WAY-100635 on polymers **P(WAY)**, **P(BL)**, and **P(BA)** in $CHCl_3$ are shown in Figure 2. The plots show greater binding to polymer imprinted with WAY-100635, **P(WAY)**, compared with polymer imprinted with benzylamine **P(BA)**. Polymer made without any template, **P(BL)**, exhibits similar affinity for WAY-100635 as polymer imprinted with benzylamine **P(BA)**.

MIP binding parameters can be estimated from the binding isotherm by a Scatchard plot,[31] a linear display of a binding isotherm where the *X*-axis represents the concentration of bound analyte and the *Y*-axis is the ratio of bound to free analyte concentration. The mean association constant (K_a) and number of binding sites (N_t) were calculated from a linear regression

Scheme 3. Synthesis of Molecular Imprinted Polymer for WAY-100635



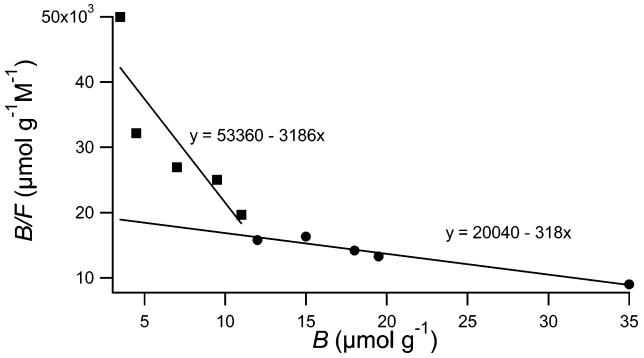


Figure 3. Scatchard plot for WAY-100635 on **P(WAY)**.

of the Scatchard plot from the expression $B/F = K_aN_t - K_aB$, where B is the concentration of bound analyte and F is the concentration of free analyte. The analysis for imprinted polymer **P(WAY)** is displayed in Figure 3.

The plot in Figure 3 exhibits a bimodal distribution of binding sites with two regions that can be fitted by linear regression. This analysis reveals the MIPs have two sets of sites with differing affinities.[31] The highest-affinity sites represented by a linear regression of the first five points in Figure 3 exhibit an estimated mean K_a of 3186 M^{-1} and number of binding sites N_t of 17 μmol g^{-1}. The lower-affinity sites are represented by the latter five points and demonstrate an estimated mean K_a of 318 M^{-1} and number of binding sites N_t of 63 μmol g^{-1}. Combining the high- and low-affinity binding sites gives a total of 80 μmol g^{-1}. The theoretical total binding sites computed from the amount of template WAY-100635 per gram of polymer is 56 μmol g^{-1}, less than that determined from the Scatchard plot. This is justified by the presence of excess methacrylic acid in the polymer formulation, which will contribute to the formation of low-selectivity binding sites.

The binding and selectivity of WAY-100635 and its analogues were also evaluated from their performance in HPLC experiments with MIP and control stationary phases. Analysis was performed by injecting 5 μL of 1 mM solutions of WAY-100635 and its analogues onto the polymeric supports using a mobile phase of 2% isopropanol in $CHCl_3$ at 0.5 mL/min. When less polar eluent was used, peaks were too broad for an accurate determination of retention times. Measurements were done in triplicate. Figure 4 depicts a chromatogram of analyte WAY-100635 on imprinted polymer **P(WAY)** and control polymer **P(BL)**. Retention times were marked as the points of absorbance maxima. Solutions of WAY-100635 and its analogues were injected onto HPLC columns containing MIP stationary phases imprinted for WAY-100635. Corrected retention volumes (capacity factors, k') were calculated against a void volume marker (acetone).[32] Imprinting factors were calculated from the ratios of k' values on imprinted and nonimprinted polymers ($I = k'_{imp}/k'_{con}$), where I is the imprinting factor and k'_{imp} and k'_{con} are the capacity factors for analytes on the imprinted polymer **P(WAY)** and the control polymer **P(BL)**, respectively. The imprinting factor can be viewed as a measure of the selectivity for the analytes for the imprinted polymer over the control polymer and thus is a measure of MIP affinity and selectivity.

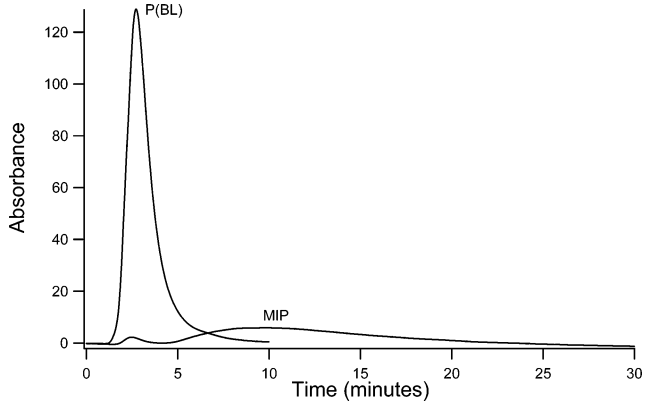


Figure 4. Chromatogram of WAY-100635 on MIP **P(WAY)** and control **P(BL)** at a flow rate of 0.5 mL/min in 2% isopropanol in $CHCl_3$.

Table 2. Chromatographic Analysis on **P(WAY)** and **P(BL)** at a Flow Rate of 0.5 mL/min in 2% Isopropanol in $CHCl_3$

compound	substituent	P(WAY) (k')	P(BL) (k')	imprinting factor (I_{org})
2	N.A.	0.2	0.2	1.0
WAY−100635	cyclohexyl	3.4	0.3	**11.3**
5	adamantyl	2.6	0.6	4.3
6	4-iodophenyl	2.7	0.4	6.7
7	phenyl	3.1	0.4	7.7
8	4-propylphenyl	3.8	0.5	7.6
9	4-pentylphenyl	1.7	0.4	4.2
10	4-heptylphenyl	1.6	0.4	4.0
11	4-fluorophenyl	2.8	0.5	5.6
12	2-furoyl	3.8	0.6	6.3

The template molecule WAY-100635 proved to be the best retained on the MIP support, all other derivatives have lower affinities for the MIP, and neither WAY-100635 nor its analogues showed any affinity for the nonimprinted **P(BL)** support (Table 2). Variations of the amide substituent at the *N*-(pyridine-2-yl) position (N2) were observed to elicit changes in the capacity factors. As the benzamido analogues **7**, **8**, **9**, and **10** get larger, a drop-off in the imprinting factor of approximately 2-fold is observed. The largest substituent change is found with the 4-heptylphenyl derivative **10**. This compound elicits the lowest imprinting factor of 4.0, while the much smaller 4-propylphenyl derivative **8** has a larger imprinting factor (7.6). Another interesting observation is that, in the absence of an amido substituent as in analogue **2**, there is no affinity for the imprinted polymer (imprinting factor of unity).

MIP chromatography columns have been shown to be successful using a variety of eluents, including aqueous mobile phases.[33,34] Their ability to operate under a range of conditions allows them to be useful for the analysis of a variety of molecules. Analyte binding to MIPs is a combination of specific and nonspecific interactions that can be attenuated by solvent.[33,35,36] In an aqueous-based eluent, nonspecific hydrophobic interactions are increased, and electrostatic/hydrogen-bonding interactions within the MIP binding site are diminished. To study

(30) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31−49.
(31) Shea, K. J.; Spivak, D.; Sellergren, B. *J. Am. Chem. Soc.* **1993**, *115*, 3368−3369.
(32) The capacity factor (k') is defined as $k' = [(V(t) - V(0))/V(0)]$, where $V(t)$ is the retention volume and $V(0)$ is the dead volume or the retention volume of a nonbinding substrate.
(33) Spivak, D.; Gilmore, M. A.; Shea, K. J. *J. Am. Chem. Soc.* **1997**, *119*, 4388−4393.
(34) Baggiani, C. In *Molecularly Imprinted Materials*; Mingdi, Y., Ramstrom, O., Eds.; Marcel Dekker: New York, 2005; pp 517−552.
(35) Kim, H.; Guiochon, G. *Anal. Chem.* **2005**, *77*, 2496−2504.
(36) Sellergren, B. *J. Chromatogr., A* **2001**, *906*, 227−252.

Table 3. Chromatographic Analysis on **P(WAY)** and **P(BL)** Supports at a Flow Rate of 1 mL/min in 7:3 ACN/10 mM KH_2PO_4 + K_2HPO_4 (aq, pH 5.8)

compound	substituent	P(WAY) (k')	P(BL) (k')	imprinting factor (I_{aq})
2	N.A.	0.4	0.3	1.3
WAY−100635	Cyclohexyl	5.5	1.9	**2.9**
5	adamantyl	6.1	2.8	2.2
6	4-iodophenyl	4.4	1.7	2.6
7	phenyl	5.0	1.9	2.6
8	4-propylphenyl	5.9	2.8	2.1
9	4-pentylphenyl	7.8	4.0	2.0
10	4-heptylphenyl	9.9	5.3	1.9

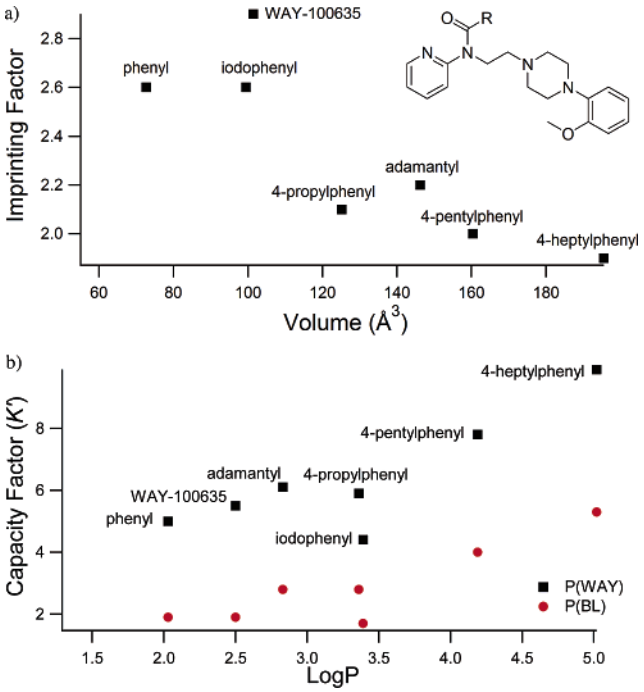


Figure 5. (a) Effect of van der Waals volume of the *N*-(pyridine-2-yl) amide substituent on the imprinting factors from an aqueous eluent (1 mL/min in 7:3 ACN/10 mM KH_2PO_4 + K_2HPO_4 (aq, pH 5.8)). (b) Effect of the lipophilicity (log P) on the capacity factors from an aqueous eluent.

the effect of solvent on binding, a chromatographic analysis was performed in an aqueous-based mobile phase. After surveying a variety of conditions, a 7:3 ACN/10 mM KH_2PO_4 + K_2HPO_4 (aq, pH 5.8) mobile phase at a 1 mL/min flow rate was found to provide the best results (Table 3). **P(WAY)** was shown to have greater affinity for WAY-100635 and its analogues over non-imprinted polymer **P(BL)** with WAY-100635 proving the most retained on the imprinted polymer with an imprinting factor of 2.9. Interestingly, *higher* capacity factors were observed on the imprinted and non-imprinted polymers in the aqueous mobile phase compared to those in the organic mobile phase. This effect is attributed to the polymer's behavior as a reverse-phase support where retention is based on, among other things, dispersive interactions between polymer and analyte.

The imprinting effect was observed to decrease with an increase in volume of the *N*-(pyridine-2-yl) amide substituents. This may be visualized in a plot of the van der Waals volume of the *N*-(pyridine-2-yl) amide substituents against the imprinting factor (Figure 5a). van der Waals volumes were calculated using Connolly's molecular surface method to provide the solvent-excluded volume which approximates the van der Waals volume.[37] The imprinting factor was found to decrease with an increase in the volume of the substituent. The 4-heptylphenyl group is the largest substituent and produces the lowest imprinting factor (1.9). This result demonstrates that the imprinted polymer's ability to discriminate between WAY-100635 analogues is based at least in part on differences in the steric bulk of the *N*-(pyridine-2-yl) amide substituents and in part on nonspecific dispersive forces.

In the reverse-phase (aqueous) chromatography mode, capacity factors were observed to increase with increasing lipophilicity. Figure 5b reveals a continuous increase of capacity factor with an increase in the calculated octanol−water partition coefficient (log P) of the *N*-(pyridine-2-yl) amide substituent. The largest and most lipophilic 4-heptylphenyl derivative **10**

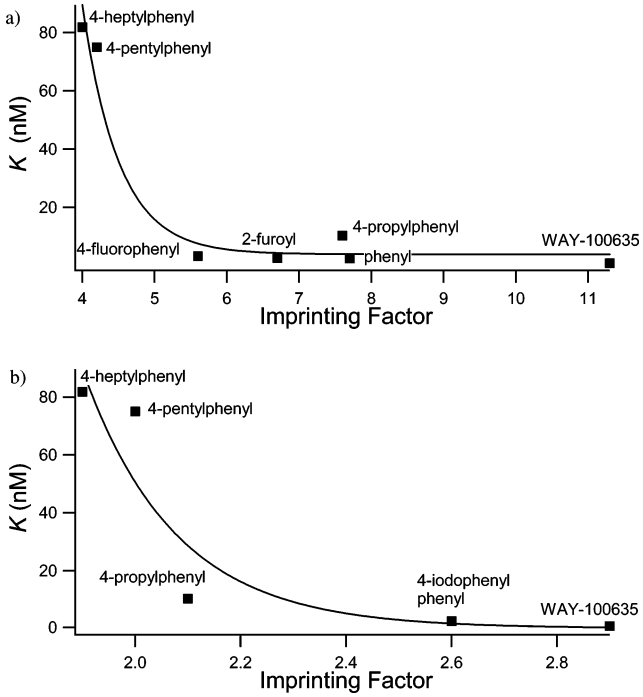


Figure 6. Relationship between 5-HT_{1A} binding affinity and imprinting factors: (a) 1 mL/min in 7:3 ACN/10 mM KH_2PO_4 + K_2HPO_4 (aq, pH 5.8). (b) 0.5 mL/min in 2% isopropanol in $CHCl_3$.

Table 4. Imprinting Factors and 5-HT_{1A} Receptor Binding Affinities for WAY-100635 and Analogues

compound	substituent	imprinting factor (I_{org})[b]	imprinting factor (I_{aq})[c]	K_i (nM)[a]
2	N.A.	1.0	1.3	N.A.
WAY−100635	cyclohexyl	**11.3**	**2.9**	0.84
5	adamantyl	4.3	2.2	*N.A.*
6	4-iodophenyl	6.7	2.6	2.6
7	phenyl	7.7	2.6	2.5
8	4-propylphenyl	7.6	2.1	10.3
9	4-pentylphenyl	4.2	2.0	75.0
10	4-heptylphenyl	4.0	1.9	81.8
11	4-fluorophenyl	5.6	*N.A.*	3.3
12	2-furoyl	6.3	*N.A.*	*N.A.*

[a] Inhibition constants on the binding of [^{125}I]-(R)-(+)-*trans*-8-OH-PIPAT to rat hippocampal homogenates.[29] [b] 2% isopropanol in $CHCl_3$ at a flow rate of 0.5 mL/min. [c] 7:3 ACN/10 mM KH_2PO_4 + K_2HPO_4 (aq, pH 5.8) at a flow rate of 1 mL/min.

(37) Connolly, M. L. *J. Am. Chem. Soc.* **1985**, *107*, 1118−1124.

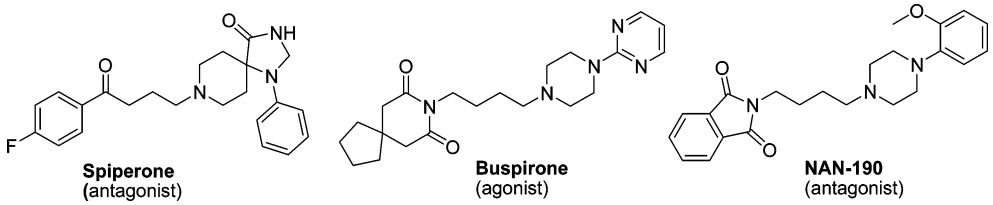


Figure 7. 5-HT_{1A} receptor high affinity agonist and antagonists.

showed the highest capacity factor on both imprinted and non-imprinted polymer. The log P is a measure of the hydrophobicity and hydrophilicity of a substance. The increased affinity for both imprinted and non-imprinted polymers with increasing lipophilicity may be attributed to nonspecific dispersive interactions, which come into play in the aqueous mobile system. It is important to note, however, that the magnitude of this trend for the non-imprinted control polymer **P(BL**) is significantly less.

The preceding chromatographic results are indicative of the contribution of amide substituents at the *N*-(pyridine-2-yl) position for retention on the imprinted polymer **P(WAY)**. Importantly, changes at this position also elicit large variations in the binding affinities for the 5-HT_{1A} receptor. The ability of the imprinted polymer to discriminate between these subtle differences adds credibility to employing imprinted polymers in the screening of libraries of ligands. A point of interest is to discern what other similarities the imprinted polymer **P(WAY)** bears with the native receptor 5-HT_{1A}.

The binding interactions that take place between WAY-100635 and the imprinted polymer and 5-HT_{1A} receptor are due to a combination of intermolecular forces. These include ionic interactions, hydrogen bonds, and/or van der Waals forces. Binding affinity is related to the Gibbs free energy of binding, ΔG, which is composed of enthalpic and entropic contributions ($\Delta G = -RT \ln K_i = \Delta H - T\Delta S$). The enthalpic contribution is comprised of van der Waals, dipole−dipole, hydrogen-bonding, and electrostatic interactions between the ligand and protein. The binding of a ligand to an imprinted polymer relies on similar interactions, although they do not share the same *specific* interactions. Nevertheless, since recognition is a composite of attractive interactions based upon the host and guest, there is no fundamental reason why parallels may not be observed.

Affinities are based on structural features of both ligand and receptor. As a consequence, variations in binding affinities can be related to deviations in ligand structure. A comparison between the forces important for ligand binding to the 5-HT_{1A} receptor and imprinted polymer can be used as a measure of how well a mimic of the receptor the imprinted polymer provides. A plot of 5-HT_{1A} receptor binding affinities against the imprinting factors for WAY-100635 and its analogues reveals such a relationship (Figure 6 and Table 4). The figures show a decrease in the imprinting factor in both organic- and aqueous-based eluents with an increase in the 5-HT_{1A} binding affinity. Sterically demanding analogues such as 4-pentylphenyl **9** and 4-heptylphenyl **10** show low affinities for both the 5-HT_{1A} receptor and imprinted polymer. WAY-100635 and other similarly sized derivatives show similar 5-HT_{1A} affinities while having moderate to high affinity for the imprinted polymer. From this we gather that a size constraint exists in both 5-HT_{1A} receptor and imprinted polymer **P(WAY)**. The imprinted polymer is able to discriminate between analogues having a 20-fold difference in affinity. Regardless of any electrostatic similarities with WAY-100635, analogues that are too large for the MIP and 5-HT_{1A} binding sites are poorly retained. From the available data, changes to the amide substituents at the *N*-(pyridine-2-yl) position of WAY-100635 elicit similar binding responses from the imprinted polymer **P(WAY**) and the 5-HT_{1A} receptor. This leads us to believe that **P(WAY)** mimics the binding-site shape and volume of the 5-HT_{1A} receptor.

Table 5. Chromatographic[a] and 5-HT_{1A} Receptor Affinities for Aryl Piperazine Antagonists

compound	P(WAY) (k')	P(BL) (k')	imprinting factor (I)[b]	K_i (nM)[b]
spiperone[39]	8.1	2.9	2.8	101[c]
buspirone[39]	2.6	1.5	1.7	14[c]
NAN-190[38]	3.4	1.0	3.4	0.6[c]
WAY-100635[29]	3.4	0.3	11.3	0.84[d]

[a] 2% isopropanol in $CHCl_3$ at a flow rate of 0.5 mL/min. [b] Inhibition constants on the binding of [^{3}H]5-hydroxyltryptamine to rat frontal cortex. [c] [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) to rat hippocampal homogenates. [d] [^{125}I]-(R)-(+)-*trans*-8-OH-PIPAT to rat hippocampal homogenates.

A variety of aryl piperazines are known to bind to the 5-HT_{1A} receptor as antagonists and agonists. They share the similar motif of an aryl piperazine and a rigid aryl segment separated by a flexible linker. We were interested in examining the imprinted polymer **P(WAY)** for the ability to distinguish aryl piperazines which were not simple analogues of the template WAY-100635 but were also potent binders to the 5-HT_{1A} receptor. Recognition by the imprinted polymer for such analogues would demonstrate homology between 5-HT_{1A} antagonists and its ability to recognize less obvious similarities between WAY-100635 and its analogues, parallel to the 5-HT_{1A} receptor. For this study we obtained the imprinting factors (chromatographic analysis on **P(WAY)** and **P(BL)** with a 2% isopropanol in $CHCl_3$ mobile phase) of spiperone, buspirone, and NAN-190 (Figure 7). Spiperone and NAN-190 are antagonists, and buspirone is an agonist of the 5-HT_{1A} receptor.

NAN-190 is the most similar to WAY-100635, sharing a common methoxy phenyl piperazine motif and antagonist behavior. NAN-190 is also the most potent, displaying high 5-HT_{1A} affinity ($K_i = 0.6$ nm).[38]A chromatographic analysis of these 5-HT_{1A} aryl piperazines on imprinted and control polymer supports show that the imprinted polymer is able to recognize aryl piperazines that are not simple analogues of the template (Table 5), with NAN-190 having the highest affinity with an imprinting factor of 3.4. Buspirone proved to be the least retained by the imprinted polymer, with an imprinting factor

(38) Raghupathi, R. K.; Rydelek-Fitzgerald, L.; Teitler, M.; Glennon, R. A. *J. Med. Chem.* **1991**, *34*, 2633−2638.
(39) Titeler, M.; Lyon, R. A.; Davis, K. H.; Glennon, R. A. *Biochem. Pharmacol.* **1987**, *36*, 3265−3271.

of 1.7. Their affinities for the imprinted polymer, however, are not as high as those of the WAY-100635 analogues.

The data indicate a relationship between the 5-HT_{1A} dissociation constants of the piperazine analogues and their imprinting factors. The most strongly retained NAN-190 is also the strongest 5-HT_{1A} antagonist, with an inhibition constant of 0.6 nM. Spiperone has a larger imprinting factor (2.8) than buspirone (1.7). This does not correlate with the inhibition constants, as buspirone shows greater affinity for the 5-HT_{1A} receptor at 14 nM than spiperone, which has an inhibition constant of 101 nM. Buspirone however is an agonist. Agonist and antagonist characters are dependent on differences in the binding interactions with the receptor. The MIP, which is imprinted for an antagonist, may have enhanced interactions with antagonists over agonists. This offers additional support for the position that imprinted polymers function as surrogates for native receptors.

Molecular Modeling Studies. Molecular modeling has been successfully employed to study the interaction between functional monomers and imprinting molecules.[40−42] Although MIPs comprise a collection of heterogeneous binding sites differing in affinity,[43] molecular modeling can be used to study possible arrangements of complementary functionality that contributes importantly to the high fidelity sites. Functional monomers can be ligated to template/analogue molecules at possible contact points and their geometry optimized. This can provide insight into the possible binding interactions within the MIP cavity and aid the analysis of binding data. This methodology was applied to the template WAY-100635 in an effort to identify possible MIP binding-site interactions. This model is limited to the hydrogen-bonding interaction between analyte and the functional monomer (MAA) and does not take into consideration the effect the volume of the MIP binding site has on affinity. The model was used to analyze the binding of 5-HT_{1A} antagonists NAN-190, buspirone and spiperone which are similar in volume to WAY-100635. They all share the common feature of a piperazine nitrogen which is believed to be integral for binding. However, other structural similarities are not as easily distinguished.

An ionic interaction between an aspartate residue of the 5-HT_{1A} receptor and the protonated piperazinyl nitrogen (N3) of antagonists has been considered crucial and via a long range Coulombic intermolecular interaction has been proposed as the first step in the binding-site recognition process.[44] This interaction is presumed to be followed by the formation of a charge-reinforced hydrogen bond. Analogously, interaction between the piperazinyl nitrogen (N3) and methacrylic acid in the prepolymer is presumed to result in a hydrogen bond between the imprinted polymer **P(WAY)** and WAY-100635.

The electrostatic potential for WAY-100635 was calculated in an effort to establish the strongest points of contact with the MIP **P(WAY)** binding site carboxylates. This was done using a semi-empirical PM3 method from the Spartan 2002 software package.[45,46] The molecular electrostatic potential is the potential energy of a proton at a particular location near a molecule. It

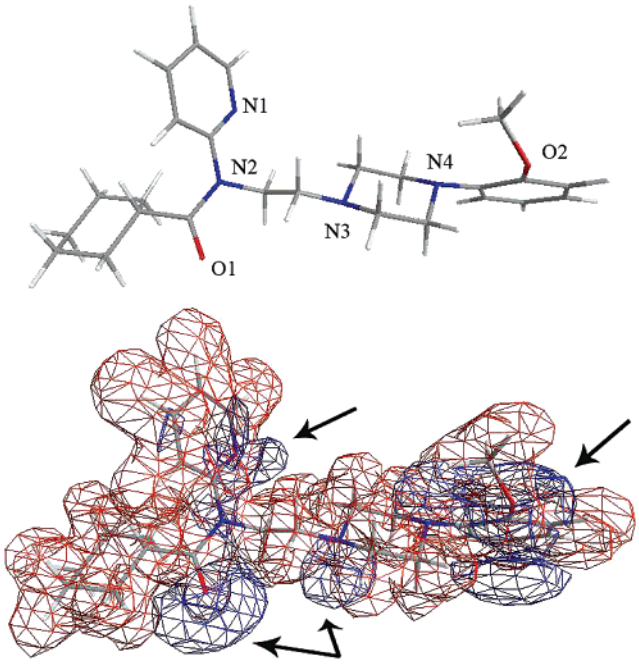


Figure 8. Molecular models of WAY-100635 with electrostatic potential mapping. Attraction for positive charge is represented by blue, and repulsion is represented by red.

represents the attraction between the molecule and a proton. The electrostatic potential is useful in rationalizing the interactions between molecules and molecular recognition processes because electrostatic forces are primarily responsible for long-range interactions between molecules. A map of the electrostatic potential on the molecular surface of WAY-100635 is shown in Figure 8. Regions attracted to a proton are signified by blue, and areas that repel a proton are red. Attractive forces between WAY-100635 and methacrylic acid in the MIP will occur at the regions colored in blue. These comprise regions around the nitrogen and oxygen heteroatoms and the methoxy phenyl ring.

Models were built ligating three methacrylic acid molecules to WAY-10635 using the Spartan 2002 software package. Methacrylic acid was first hydrogen bonded to the piperazine nitrogen (N3) and the *N*-(pyridine-2-yl) amide oxygen (O1). The third methacrylic acid was hydrogen bonded to either the pyridinyl nitrogen (N1) as in Figure 9a or the phenyl methoxy oxygen (O2) as illustrated in Figure 9b. Their geometries were then optimized with the PM3 semi-empirical method and are illustrated in Figure 9. From this we gathered that the complex where the third methacrylic acid was hydrogen bonded to the methoxyl phenyl oxygen (O2) was more favored, with a calculated heat of formation of −296.18 kcal/mol compared to the −292.47 kcal/mol that was obtained for the pyridinyl nitrogen (N1) hydrogen-bonded structure. It is proposed that an idealized MIP binding site could include three methacrylic acid hydrogen-bonding contacts to the piperazinyl nitrogen (N3), *N*-(pyridine-2-yl) amide oxygen (O1), and the methoxy phenyl oxygen (O2).

The methacrylic acid molecules in Figure 9b were fixed into position to mimic the WAY-100635 MIP binding site. The WAY-100635 molecule was deleted, and 5-HT_{1A} antagonists NAN-190, spiperone and agonist buspirone were inserted into

(40) Wu, L.; Li, Y. *J. Mol. Recognit.* **2004**, *17*, 567−574.
(41) Wu, L.; Sun, B.; Li, Y.; Chang, W. *Analyst* **2003**, *128*, 944−949.
(42) Takeuchi, T.; Dobashi, A.; Kimura, K. *Anal. Chem.* **2000**, *72*, 2418−2422.
(43) Umpleby, R. J., II; Bode, M.; Shimizu, K. D. *Analyst* **2000**, *125*, 1261−1265.
(44) Strader, C. D.; Sigal, I. S.; Candelrone, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. *J. Biol. Chem.* **1988**, *263*, 10267−10271.
(45) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209−220.
(46) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 221−264.

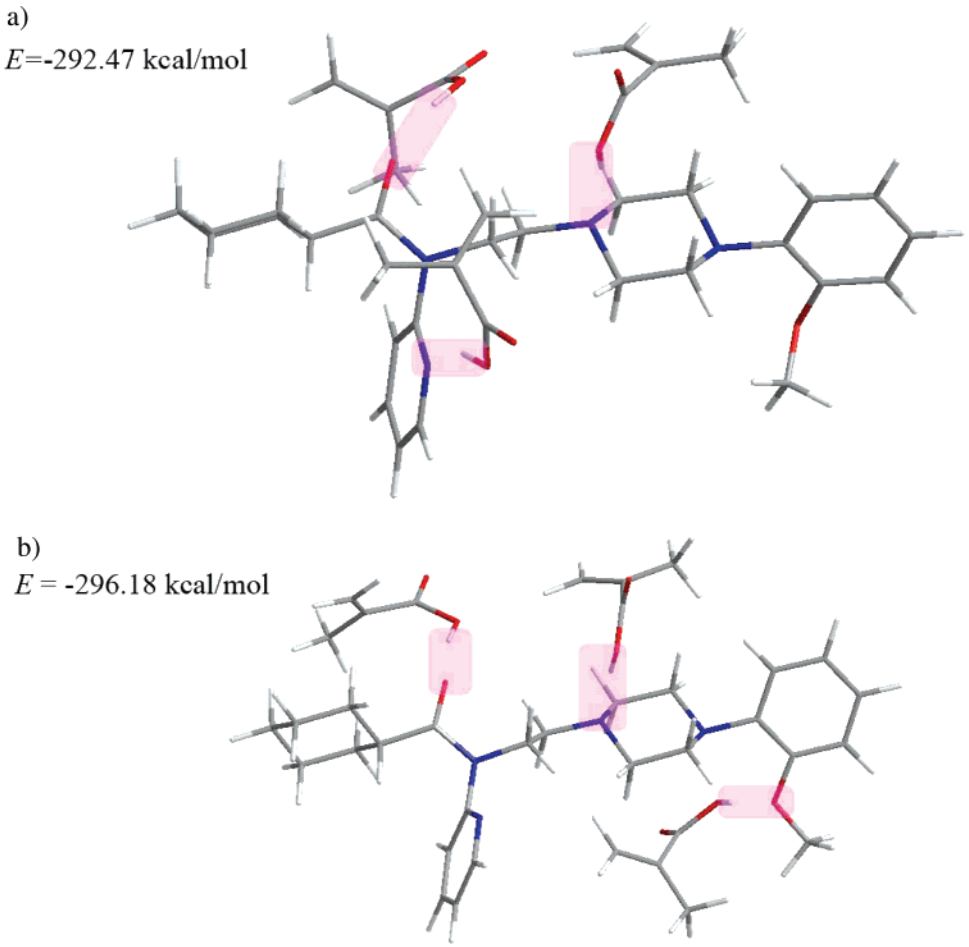


Figure 9. Molecular model of three methacrylic acid molecules bound to WAY-100635 heteroatoms: (a) N1, N3, and O1; (b) N3, O1, O2.

the model. When inserted, all models had the piperazinyl nitrogen hydrogen bonded to a methacrylic acid molecule. All molecules were oriented in the binding cavity with the aryl piperazine in the same region in a manner similar to that of WAY-100635 in Figure 9b. With the methacrylic molecules in fixed positions, the geometries were then optimized using the PM3 semi-empirical method, the result of which can be found in Figure 10.

All 5-HT_{1A} antagonists are able to establish multiple contacts with methacrylic acid functional monomers in the proposed WAY-100635 MIP cavity. Each has electronegative atoms in close proximity to acidic hydrogen of methacrylic acid. Most notable are NAN-190 and spiperone which show contact with all three methacrylic acids. In addition to the piperazine nitrogen, NAN-190 exhibits hydrogen bonding with the phthalimide oxygen and methoxy phenyl oxygen (Figure 10a). Spiperone is observed having hydrogen bonds with the methacrylic acids of the WAY-100635 MIP cavity at its piperazine nitrogen, aryl ketone oxygen, and cyclic amide oxygen. Interestingly, in the optimized structure the agonist buspirone could have only two hydrogen bonds with the methacrylic acids of the proposed WAY-100635 MIP cavity, one at the piperazine nitrogen and the other at imide oxygen.

It is interesting to note that both NAN-190 and spiperone, which were observed to be capable of three hydrogen bonds in the proposed WAY-100635 MIP cavity, show greater retention on the MIP chromatography column **P(WAY)** than buspirone (Table 5). It is possible that the increased imprinting factors on MIP **P(WAY)** for NAN-190 and spiperone compared to that for buspirone may be due to the additional ability to enjoy hydrogen bonding at the aryl portion of the molecule.

Similarities between the proposed MIP binding model and 5-HT_{1a} receptor binding site are apparent. In recent homology modeling of the 5-HT_{1a} receptor in addition to ionic interaction between the carboxylic acid side chain of Asp3.32 and the nitrogen of the piperazine moiety, other essential interactions were hydrogen bonding between hydrogen bond acceptors of the ligand with Ser5.42 and Asn7.39.[47] Inspection of the 5-HT_{1a} receptor binding to NAN-190 (Figure 11) shows similarities with NAN-190 bound to our proposed MIP binding model (Figure 10a). Both binding models show interactions with the imide carbonyl, piperazine nitrogen, and phenyl methoxy oxygen atoms of NAN-190.

Summary

In conclusion we have developed a synthetic MIP receptor for the recognition of WAY-100635 and its analogues. The binding sites are heterogeneous in nature, as expected for a noncovalent imprinted polymer. A Scatchard analysis shows selective binding sites with a mean association constant of 3186 M^{-1} and nonselective binding sites with a mean association constant of 318 M^{-1} in chloroform. In analogy with the native receptor there is a steric constraint on the analogues within the

(47) Nowak, M.; Kolaczkowski, M.; Pawlowski, M.; Bojarski, A. J. *J. Med. Chem.* **2006**, *49*, 205−214.

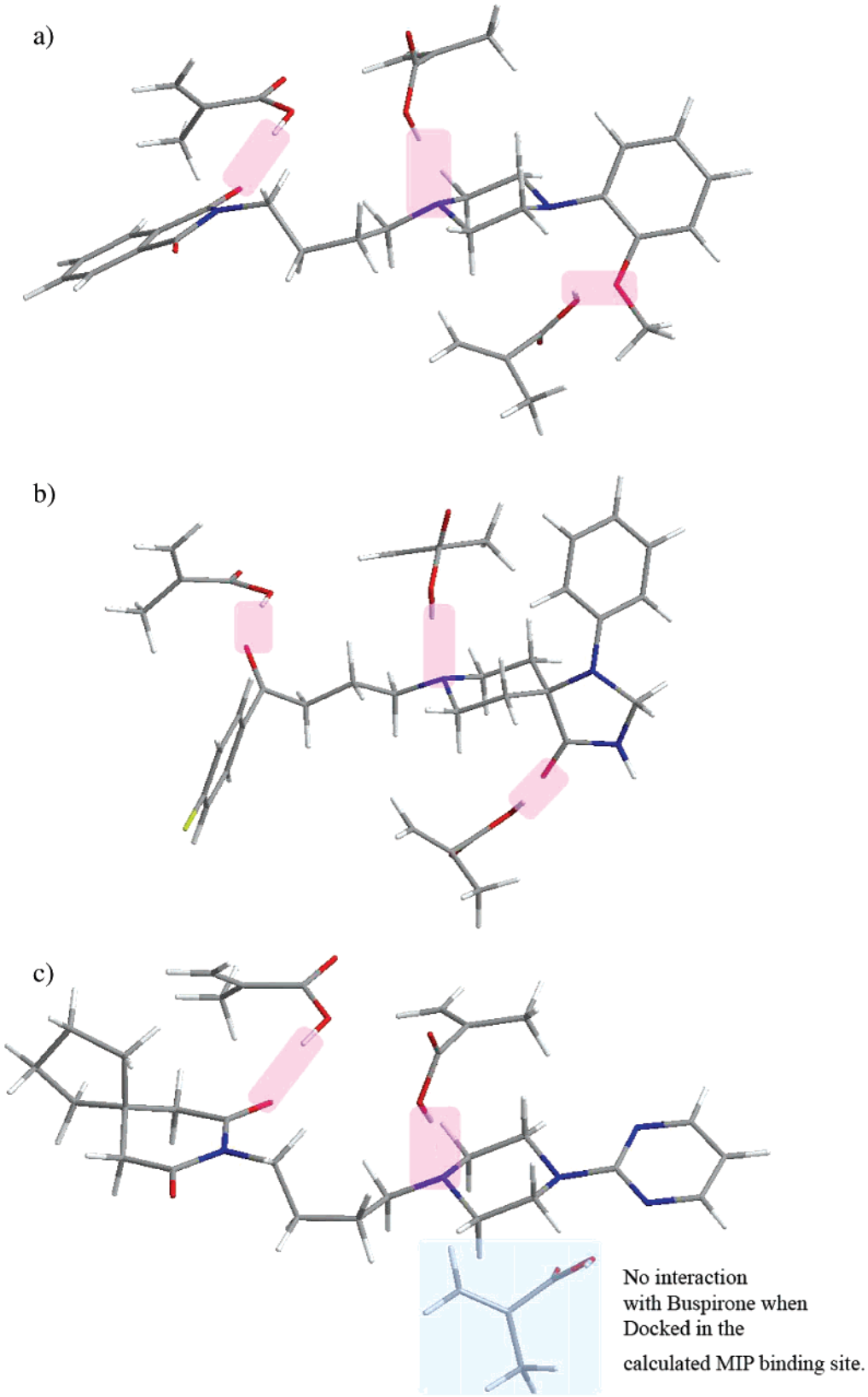


Figure 10. Models of 5-HT1A antagonists bound to three methacrylic acids in WAY-100635 MIP. Hydrogen-bonding interactions are highlighted. (a) NAN-190; (b) spiperone; (c) buspirone.

imprinting cavity, and as *N*-(pyridine-2-yl) amide substituents became larger, a drop-off in affinity for the imprinted polymer was observed. We have also shown that the imprinted polymer shows affinity for aryl piperazines which are not direct analogues of the imprinting template. A relationship was observed to exist between the 5-HT_{1A} receptor and MIP affinities, with both showing limited tolerance for variations at the amino pyridine position in a series of benzamido analogues of WAY-100635.

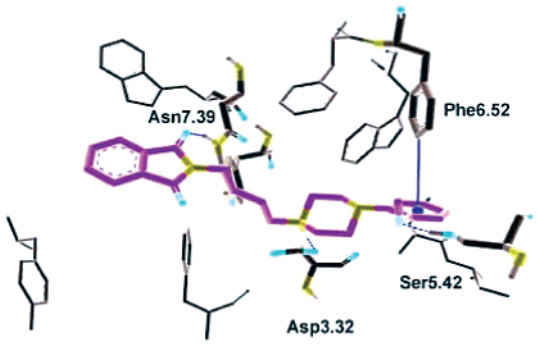


Figure 11. Calculated interactions of NAN-190 bound to the 5-HT_{1a} binding site.[47]

These results support the viability of imprinting polymers as surrogates for native receptors in drug discovery.

Experimental Section

General Procedures. Compounds **1**−**12** were prepared in a manner similar to those of documented procedures.[29,48] High-pressure liquid chromatography (HPLC) experiments were performed on a HP 1100 dual solvent delivery system. Molecular modeling was done using the Spartan 2002 software package.

General Procedure for Molecularly Imprinted Polymer Synthesis. A solution of ethylene glycol dimethacrylate (6.78 g, 34.21 mmol), methacrylic acid (0.41 g, 4.77 mmol), and dimethoxy-2-phenyl acetophenone (0.103 g, 0.4 mmol) in chloroform (9.3 mL) was deoxygenated using nitrogen gas for 5 min. Additional deoxygenated solvent was added to bring the volume of the solution to its initial level. The appropriate template (0.4 mmol) was added and solution further deoxygenated. The polymerizations were initiated photochemically by a mercury arc UV light source at 5 ± 2 °C and allowed to proceed for 24 h under constant irradiation. After being crushing, the polymers were extracted with methanol for 24 h and then dried under vacuum. The polymers were further crushed using a mortar and pestle and sieved to isolate 38−125 μm particles for use in batch rebinding studies.

General Procedure for Batch Rebinding Experiments with Molecularly Imprinted Polymers. A stock solution of 5 mM WAY-100635 in solvent was prepared. This solution was diluted with 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, and 2 mM solutions of solvent. One milliliter of each WAY-100635 solution was then added to a 2.0-mL screwcap HPLC vial containing either no polymer or 20 mg of polymer **P(WAY)**, **P(BA)**, or **P(BL)** (38−125 μm size range). The vials were then shaken for 24 h and the unbound WAY-100635 concentrations quantified using HPLC analysis. This concentration is labeled as F and the concentration of bound WAY-100635 to polymer is designated B and is computed by taking the difference between the total concentration of WAY-100635 (T) and F (i.e., $B = T - F$).

General Procedure for Chromatographic Experiments. Polymer particles (25−38 μm) were slurry packed into stainless steel columns (length: 100 mm, internal diameter: 4.6 mm). Once packed, the columns were washed using an eluent of 70/30 ACN/H_2O until a stable baseline was observed. Chromatographic experiments were then performed using these columns with the Agilent 1100 system.

Acknowledgment. We are grateful to Wyeth Pharmaceuticals, the NIH, and University of California, Irvine for financial support.

Supporting Information Available: Complete ref 28. Data for binding isotherm of **P(WAY)**, **P(BA),** and **P(BL)**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(48) Zhuang, Z.; Kung, M.; Kung, H. F. *J. Med. Chem.* **1994**, *37*, 1406−1407.