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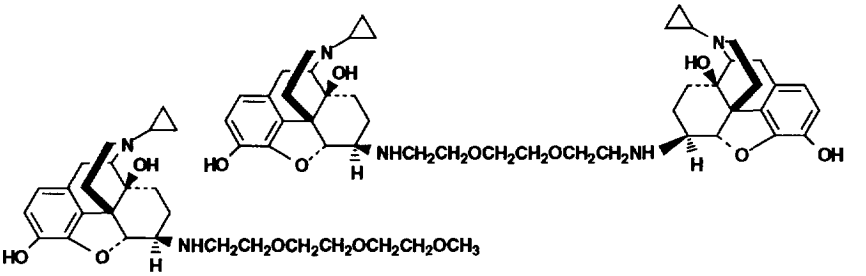
BIVALENT INDOLES EXHIBITING SEROTONERGIC BINDING AFFINITY

Karen L. LeBoulluec*, Ronald J. Mattson, Cathy D. Mahle,
Rachel T. McGovern, Henry P. Nowak, and Anthony J. Gentile

Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut 06492-7660 USA.

Abstract. A series of bis-5-carboxamidoindoles were prepared and examined in a number of serotonin assays ($5HT_{1A}$, $5HT_{1D}$, $5HT_{1E}$, and 5HT UT).[1] They were found to exhibit good affinity for the $5HT_{1A}$ and $5HT_{1D}$ receptor subtypes and to inhibit the 5HT uptake sites. Optimal $5HT_{1D}$ potency was achieved for bivalent analogs **5f** and **5g**, whose linkers spanned 7 and 8 alkyl units. Analogs with longer alkyl chain linkers (n= 10, 12), **5h** and **5i**, exhibited no selectivity for the $5HT_{1D}$ receptor over the $5HT_{1A}$ receptor.

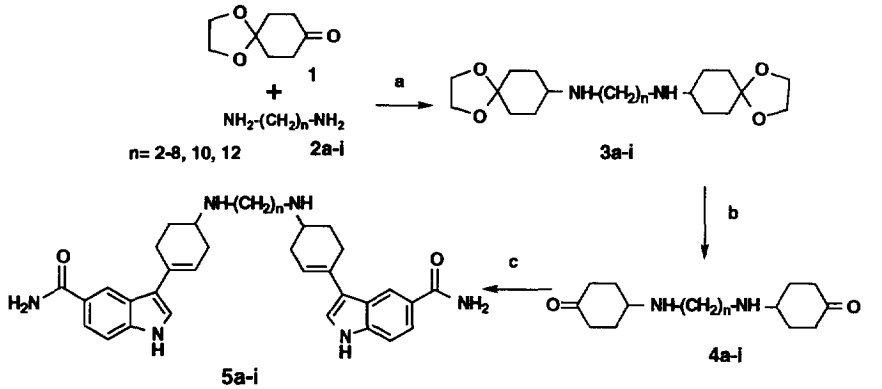
A number of bivalent ligands have been prepared and reported in the literature.[2] Many of them have been found to have enhanced activity and selectivity over their respective monomer counterparts. In particular, the use of two pharmacophores in a single ligand has been extensively investigated in the opioid area. For example, there has been much interest in the role of bivalent ligands as molecular probes for identification of multiple opioid receptor subtypes.[3a] In particular, a potency study of bivalent ligands containing ß-naltrexamine proved that considerably greater narcotic antagonistic activity was seen for the bivalent ligands compared to their monovalent congeners. Moreover, it is conceivable that specific opiod receptors μ, κ, and δ exhibit different selectivity toward bivalent ligands depending on their bridging distances. This suggests that bivalent linker distances maybe associated with different receptors.[3b]



Several theories have been proposed to rationalize bivalent ligand activity.[3c] The theories presently used to explain the high selectivity of a bivalent ligand for a single opioid receptor subtype are (1)

simultaneous interaction of both pharmacophores with vicinal receptors occuring when spacer chain of bivalent ligand is an optimal length or (2) simultaneous interaction under conditions not favoring a univalent binding mode. Observations disclosed by Thue Schwartz[4] and Jurgen Wess[5] support these theories. They have proposed a new two domain model for the structure and function of G-protein coupled receptors. The model would allow the entrapment of one pharmacophore of a bivalent ligand leaving the tether free to position the second pharmacophore into an adjacent receptor site. Such an interaction could occur provided vicinal receptors were present and a simultaneous binding occured with the optimal chain linker. Further more, Jurgen Wess has shown using two genetically engineered transfected transmembranes in a cell that it was necessary that a dimer complex form in the plasma membrane for a response to be produced. The dimer complex hence would allow for neighboring receptor sites. These observations lend support to the theories presently accepted to explain the high selectivity of a bivalent ligand for a single receptor.

In this manuscript, we demonstrate bivalent indoles containing various diamine alkyl chain linkers bind selectively to the $5HT_{1D}$ receptor subtype. This binding selectivity was not seen for the monomer **6** ($5HT_{1A}$: IC_{50}= 1.27 nM; $5HT_{1D}$: IC_{50}= 1.98 nM).[6] The bivalent diamine linked indoles **5a-5i** were prepared in three steps starting from the appropriate diamine linker **2a-i**. Sodium triacetoxyborohydride was used in a one step condensation-reductive amination procedure of **1** with **2a-i** to yield **3a-i**. The hydrolysis of **3a-i** with 50% sulfuric acid in a 1:1 solution with tetrahydrofuran at room temperature afforded **4a-i**. The final bis-indole products **5a-5i** were obtained by condensation of **4a-i** and the 5-carboxamidoindole.

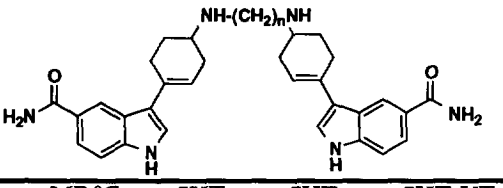


a) $NaBH(OAc)_3$/ CH_2Cl_2/ RT/ 18 hrs; b) 1:1 Solution of 50% H_2SO_4- THF/ RT/ 18 hrs; c) 5-Carboxamidoindole/ EtOH/ pyrrolidine/ reflux/ 3days

In the first stage of this study, the bivalent indoles **5a-i** were evaluated for their serotonergic affinity at the $5HT_{1A}$, $5HT_{1D}$, and $5HT_{1E}$ receptor sites.[1] They were also examined for their ability to inhibit 5HT uptake sites. The results are listed in Table 1. The 5-carboxamidoindoles **5a-i** were found to

exhibit good affinity for the $5HT_{1A}$ and $5HT_{1D}$ receptor subtype.[7] For these analogs, the optimal chain linkage was found to be seven and eight methylene units. These compounds **5f** (n=7)[8] and **5g** (n=8) displayed IC_{50}'s at the $5HT_{1D}$ site of 0.05 nM and 0.11 nM, respectively. Moreover, the selectivity over the $5HT_{1A}$ site were 36 and 19 fold, respectively. The higher homologs **5h** (n=10) and **5i** (n=12) were found to have lost selectivity for the $5HT_{1D}$ receptor subtype although its affinity remained good for both the $5HT_{1A}$ and $5HT_{1D}$ receptors. This may suggest different bivalent linker distances can discriminate different receptor subtypes.[3b] The bivalent analogs **5a-i** all exhibit good binding affinity for the 5HT uptake site and no binding affinity for the $5HT_{1E}$ receptor site. The postulate that bivalent binding may occur for such ligands is further supported by the observation that the bivalent indole **5f** binds with 40 fold greater affinity for the $5HT_{1D}$ receptor than the monomer **6**.

Table 1 : Serotonergic Affinities (IC_{50}) of Selected Bivalent Indoles.

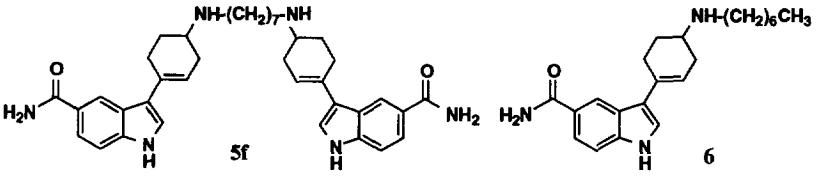


no.	n	MP °C fum. salt	$5HT_{1A}$ nM	$5HT_{1D}$ nM	5HT-UT nM	$5HT_{1E}$ nM
5a	2	265	2.0	8.0	12.6	>1000
5b	3	188-192 foam	18	27.3	NA	>1000
5c	4	200	14.5	20.3	47.76	>1000
5d	5	189-194	2.86	3.35	47.54	>1000
5e	6	176-180	2.27	0.83	33.91	>1000
5f	7	230	1.83	0.05	17.67	99[a]
5g	8	220	2.13	0.11	12.42	392
5h	10	175-180 foam	0.22	2.63	NA	>1000
5i	12	175-180 foam	0.13	0.33	NA	NA

[a] indicates 3 point data; NA denotes data not available.

In conclusion, this study lends support to the theory that bivalent ligands may exhibit greater affinity and selectivity for a specific receptor subtype over their monomer counterparts. It may also be

stated that differential receptor selectivity may be achieved by altering the length of the tether containing the two ligands. Future studies will focus on the preparation of bivalent ligands possessing a variety of linker types which retain the optimal spanning distance, as well as, determination of their intrinsic activities.



5f 6

$5HT_{1A}$ IC_{50}= 1.83nM	$5HT_{1A}$ IC_{50}= 1.27nM
$5HT_{1D}$ IC_{50}= 0.05nM	$5HT_{1D}$ IC_{50}= 1.98nM

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6) Bis-Coat $5HT_{1A}$ IC_{50}= 0.7 nM and $5HT_{1D}$ IC_{50}= 0.7 nM.
7) The 5-fluoroindole (n= 2) and the 5-cyanoindole (n= 2) dimers exhibited no $5HT_{1A}$ or $5HT_{1D}$ affinity.
8) Data for **5f**: ^1H NMR (300 MHz, DMSO-d_6) d 8.36 (s, 2H), 7.94 (br.s., 2H), 7.66 (d, 2H, J= 8.7 Hz), 7.41 (s, 2H), 7.36 (d, 2H, J= 8.5 Hz), 7.12 (br.s., 2H), 6.19 (br.s., 2H), 3.43-2.48 (m, 6H), 2.07-1.29 (m, 22H). HRMS calc for $C_{37}H_{47}N_6O_2$ (M+H): 607.3761. Found: 607.3751.

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