

Short Research Article **RESCUING ABANDONED MOLECULES**

AS Nav1.7 AND PCSK9 INHIBITORS

ABSTRACT

Academic institutions are plentiful of unexploited compounds. A cost-effective repositioning strategy from abandoned molecules coming from different research projects was applied. This strategy was based on the creation of a 2D-database then the biological assaying by the adhesion to the Lilly OIDD (Open Innovation Drug Discovery) program combining *in silico* methods and *in vitro* screening modules representing therapeutic areas of significant interest. The best results involve some molecules, that demonstrated to be active voltage-gated sodium ion channel Nav1.7 inhibitors. The inhibition of Nav1.7 is considered to be a potential mechanism for the treatment of chronic pain. Other findings refer to the inhibition of PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) synthesis, a convertase that plays a regulatory role in the metabolism of cholesterol. This work demonstrates how successful strategies can be established by mutually Academic-Industrial collaborations that can lead to the discovery and development of new medicines exploiting the groundbreaking potential emerging from academic laboratories. Abandoned molecules from various academic research projects, might be the starting point for innovative and cost-saving opportunities for both Academia and Industry.

Key words: Drug Repositioning - Database Building - Cheminformatics - In Vitro Screening - Nav1.7 - PCSK9

1. INTRODUCTION

Nowadays the drug discovery process requires a considerable effort in terms of time-consuming, cost and failure risk. The drug development takes several years due to the requirements regarding safety, efficacy and quality. Moreover, the attrition rate is another relevant aspect to be considered during the main stages of the drug pipeline (discovery, lead identification-optimization, preclinical studies and clinical trials); hundreds of thousands of molecules are discarded before the final product is obtained.

In this context it becomes reasonable to find new strategies for optimizing the available resources. Academic collections can be an excellent source to rescue or reprofile molecules. [1] exploiting at best low level of investment, reduced development timeline, recycling of resources. New technologies as computational predictions, virtual screening and HTS (high-throughput screening) methods can be powerful tools for a drug-rescuing strategy.

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The Lilly OIDD (Open Innovation Drug Discovery) screening module based on Nav1.7 aims to find a selective antagonist for this sodium channel which is expressed primarily in the peripheral sensory neurons, sympathetic ganglia. Selective antagonist of Nav1.7 channel, may be a promising therapeutic approach in the treatment of chronic pain [2]. The Lilly OIDD (Open Innovation Drug Discovery) phenotypic screening module based on PCSK9 synthesis inhibition aims to identify compounds that inhibit PCSK9 transcription using assays based on HepG2 (human hepatoma) cell lines. This protein convertase, induces the degradation of LDLR (Low Density Lipoprotein Receptor). The inhibition of PCSK9 reduces plasma levels of LDL cholesterol, increasing the function of LDL receptors that mediate the endocytosis of LDL [3]. Therefore, compounds which inhibit the PCSK9 synthesis, represent a valid approach in the treatment of hypercholesterolemia and related diseases.

2. MATERIALS AND METHODS

Abandoned molecules coming from different research projects were submitted to Lilly OIDD program [4]. Although the dataset is quite small when compared to the whole collection promising activities were found as sodium channels Nav1.7 and of PCSK9 synthesis inhibition.

2.1 Database building

The database was built by molecules selected from papers published by groups of the Department of Drug Sciences of the University of Catania. The used filtering criteria for the selection were:

- corresponding author/s from the Department of Drug Sciences of the University of Catania.
- Period 2002 – 2012.

- Origin (were selected only molecules synthesized in the Drug Sciences Department of Catania).

Finally, 38 publications were selected from which 627 molecules of interest were extracted. The drawing process was carried out on a desktop PC, CPU Intel® Core™ i7 960 3.20 GHz - RAM 16GB. Structures were sketched as 2D MDL Molfiles using Accelrys – AccelrysDraw 4.1 [5]. The main stereochemical variants for each compound were considered; therefore enantiomers and stereoisomers for molecules which have asymmetric carbons (R/S) and/or double bonds (E/Z) were also drawn, respectively. The creation of molecules libraries to build the database was carried out by Accelrys – Discovery Studio 3.5 Client [5]. libraries were built as SDF files. Finally, have been created 38 libraries containing overall 627 molecular structures.

2.2 *In Silico* screening

The *in silico* screening was performed by the automated procedure “cheminformatics structure evaluation” [6] a sequence of algorithms written by Eli Lilly & C. *In silico* evaluation has been used to filter the large structures database to the subsequent *in vitro* Screening (HTS). The overall cheminformatics processing was based on a set of rules for identifying potentially reactive or promiscuous compounds [7], and to evaluate the innovativeness and properties of analyzed structures. Finally, the algorithms were used also for evaluating the “drug-likeness” of the structures [8]. Cheminformatics structure evaluation was performed in different sequential steps which were essentially based on: substructure searching tools, filters to discard pharmaceutically undesirable motifs, molecular similarity comparison and model predictions. The molecular structures were stored encrypted in the secured area of Lilly OIDD website. The cheminformatics structure evaluation started with an automatic conversion of structures into molecular fingerprints. Only these generated bit-strings passed to the Lilly internal network for the *in silico* evaluation process. All the chemical structures submitted were totally encrypted, therefore the confidentiality of compounds was absolutely ensured.

The fingerprint generation for the molecules under study was performed through linear and circular path methods, then the fingerprints were combined with a small set of calculated molecular properties [6]. The similarity measures between two fingerprints were calculated using the Tanimoto coefficient also known as Jaccard coefficient [9]. The computational tools used during Lilly OIDD cheminformatics structure evaluation included also QSAR (quantitative structure-activity relationship) models based on

86 Tanimoto kernel function. These models have been used in a virtual screening assessment on some
87 specific targets of Lilly OIDD program.

88 **2.3 *In Vitro* screening**

89 *In vitro* evaluation was performed by the Open Innovation Drug Discovery Screening Panel. The high-
90 throughput screening were performed in the laboratories of Eli Lilly and Company, Indiana – USA. The
91 performed assays comprised several target-based and phenotypic modules. The flow of each module
92 included primary, secondary and confirmatory assays with established thresholds for progressing
93 during the module flow. The main details concerning targets, phenotypes as well the flow schemes
94 about all modules of the *in vitro* screening are available by the Lilly OIDD website [4]. The assays of
95 Lilly OIDD program have been developed according with the Assay Guidance Manual available on the
96 National Center for Biotechnology Information [10].

97 The Percentage stimulation and percentage inhibition were calculated by Equation (1) and Equation
98 (2), respectively, using the maximum (Max) and minimum (Min) response conditions for each assay:

- 99 • Equation (1): Stimulation % = $(\text{Signal} - \text{Min} / \text{Max} - \text{Min}) \times 100$
- 100 • Equation (2): Inhibition % = $1 - (\text{Signal} - \text{Min} / \text{Max} - \text{Min}) \times 100$

101 The EC50 or IC50 of tested compounds has been determined using a standard four-parameter logistic
102 and nonlinear regression analysis based on the calculated percentage activation or inhibition values.

103 Concerning the target-based module Nav1.7, the assays have been performed on HEK-293 (Human
104 Embryonic Kidney 293) cells, using the IonWorks® Quattro™ (IWQ) electrophysiology platform with
105 automated patch clamp system.

106 The primary assays to evaluate the synthesis inhibition of PCSK9 have been performed on Human
107 cell line: HepG2 (Hepatocellular carcinoma). The inhibition of non-specific secretion has been tested
108 through a Metridia Luciferase Reporter Assay [10]. The secondary assays were based on enzyme-
109 linked immunosorbent assays (ELISA) and have been performed on RPH (Primary Rat Hepatocyte)
110 cells [10].

111 **3. RESULT AND DISCUSSION**

627 compounds that were selected from 38 published works by research groups of the Drug Sciences Department, University of Catania were screened by chemoinformatics structure evaluation. 213 out of the 627 molecules passed the cheminformatics evaluation. 65 out of the 213 samples were tested by the Lilly OIDD *in vitro* screening modules.

In the PCSK9 Screening module, 3 compounds showed an inhibition of PCSK9 synthesis during the primary assays on HepG2 cell line. Table 1 summarizes both the results of primary Single Point (SP) percentage inhibition assays performed in two different concentrations (5 μ M - 50 μ M) and values of IC50 obtained through primary Concentration Response Curve (CRC) assay. In the same table, the latter IC50 values of Metridia Luciferase Reporter assay (MLR) demonstrate how the mentioned compounds have not a relevant inhibition of non-specific secretion. Table 1 summarizes also the results of secondary Single Point (SP) assays that were performed on RPH cell line in two different concentrations (20 μ M - 100 μ M) and twice duplicated for each concentration, R1 and R2. In the secondary assays, the compounds did not confirm the inhibition activity previously demonstrated on HepG2 cell line during primary assays. Due to the low values of percentage inhibition on RPH primary cell line, the mentioned compounds have not been accepted for further assays of the Lilly OIDD PCSK9 module.

Table 1. PCSK9 Screening module - Primary and Secondary assays

<u>Compound</u>	<u>Primary SP</u>	<u>Primary SP</u>	<u>Primary CRC</u>	<u>Primary MLR*</u>	<u>Secondary SP</u>	<u>Secondary SP</u>	<u>Secondary SP</u>	<u>Secondary SP</u>
	%Inhib. (5 μ M)	%Inhib. (50 μ M)	IC50 (μ M)	IC50 (μ M)	%Inhib. (20 μ M)	%Inhib. (20 μ M)	%Inhib. (100 μ M)	%Inhib. (100 μ M)
	HepG2	HepG2	HepG2	HepG2	RPH (R1)	RPH (R2)	RPH (R1)	RPH (R2)

PV0235	6,6	107,4	4,163	> 50,0	3,613	- 4,145	13,25	10,34
RG0222	5,6	71,3	0,1472	47,41	- 8,886	- 5,29	27,34	15,27
SL0134	- 30,4	98,6	6,827	33,19	7, 494	7,538	82,78	94,48

* Metridia Luciferase Reporter assay to evaluate the inhibition of non-specific secretion

The above-mentioned compounds (Fig. 1) that show an activity in the inhibition of PCSK9 synthesis, were originally synthesized for different targets. Compound **PV0235** belongs to a series of 2-substituted-4-aryl-3-quinolinecarboxylic acid derivatives which were originally synthesized as ligands of endothelin ET_A receptor [11]. Compound **RG0222** shows the best value of IC₅₀ as inhibitor of PCSK9 synthesis on HepG2 (Table 1). This compound was initially synthesized as selective ligand of α₁ adrenoceptor [12]. Finally, compound **SL0134** belongs to a series of imidazole-based ligands that were originally synthesized as inhibitors of nitric oxide synthase [13]. Even more interesting would be considered the similarity of some molecular substructures between **PV0235** and the natural compound **Berberine** (Fig. 1), an alkaloid that suppress the PCSK9 transcription [14].

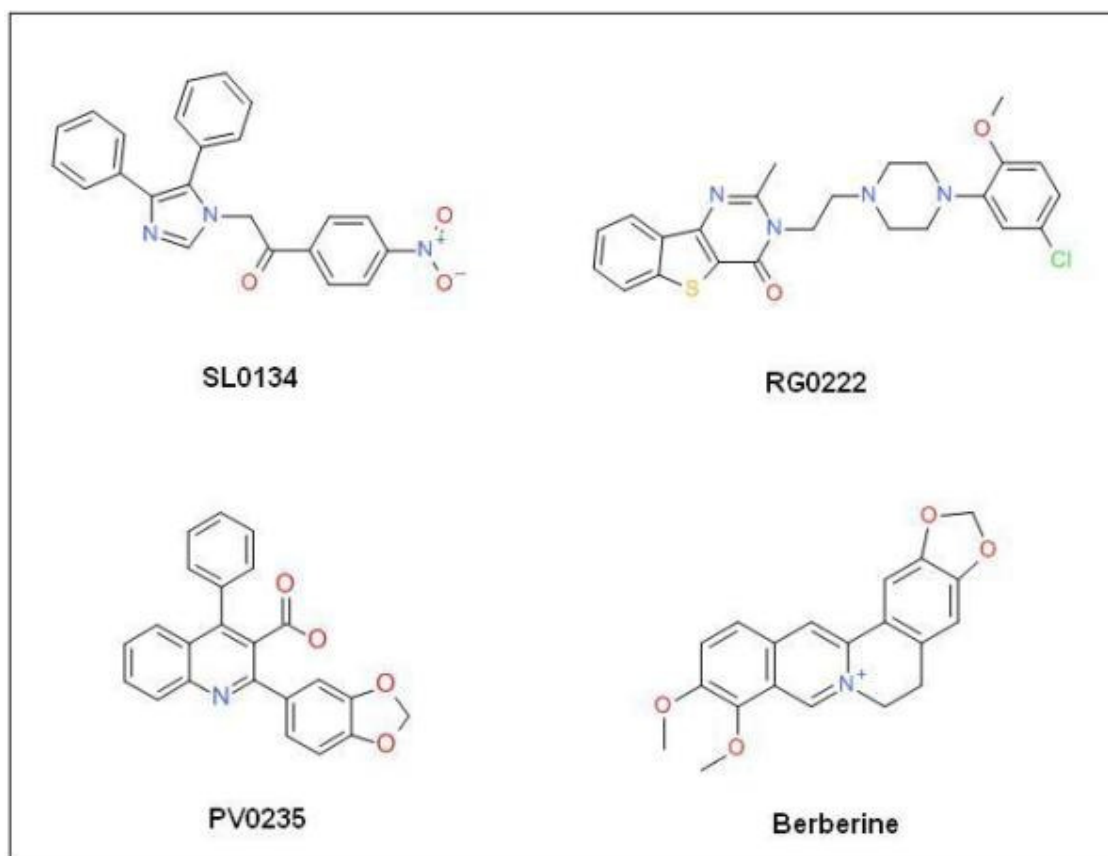


Fig. 1. Compounds under study that showed inhibition of PCSK9 synthesis; Berberine is a natural alkaloid that decrease PCSK9 expression.

The best results of our drug repositioning strategy involve the screening module based on the identification of selective Nav1.7 inhibitors. Six compounds demonstrated inhibition during primary assays on Nav1.7; the obtained values of Single Point (SP) percentage inhibition and values of IC₅₀ are shown in Table 3. The Concentration Response Curve (CRC) assays to calculate the values of IC₅₀ were duplicated R1 and R2. All six compounds demonstrated inhibition also during the secondary assay that involved Nav1.5, a different subtype of voltage-gated sodium ion channel which is expressed primarily in the cardiac muscle. The IC₅₀ values on Nav1.5 (Table 3), demonstrated a low selectivity of the compounds between the targets Nav1.7 and Nav1.5.

162 **Table 3: Nav1.7 Screening module - Primary and Secondary assays**

<u>Compound</u>	<u>Primary</u> hNav1.7 %Inhibition (3 μ M)	<u>Primary</u> hNav1.7 IC50 (μ M) R1	<u>Primary</u> hNav1.7 IC50 (μ M) R2	<u>Secondary</u> hNav1.5 IC50 (μ M) R1	<u>Secondary</u> hNav1.5 IC50 (μ M) R2
RG0159	98,9	0.4329	0.745	1.298	1.122
RG0158	91.8	1.49	1.007	2.395	2.168
RG0321	91.6	1.685	0.7944	3.291	1.096
SMA0132	69.6	4.014	1.718	1.639	1.998
RG0428	60.7	2.145	1.768	1.862	1.871
MA0110RS	60.2	3.507	4.117	5.86	4.362

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165 The best Nav1.7 inhibition activity has been demonstrated by the compounds **RG0159**, **RG0158** and
 166 **RG0321** (Fig. 2). These molecules were originally synthesized as selective ligands for α_1 -adrenoceptor
 167 subtypes [15,16]. **RG0428** (Fig. 2) belongs also to a series of ligands for α_1 -adrenoceptor subtypes
 168 [17]. Compound **SMA0132** (Fig. 2) belongs to a series of potent and selective 5-HT_{1A} Serotonin
 169 receptor ligands [18]. **MA0110RS** (Fig. 2) that was initially synthesized as selective ligand of σ
 170 receptor [19], demonstrated the lowest inhibition activity on both Nav1.7 and Nav1.5 sodium
 171 channels.

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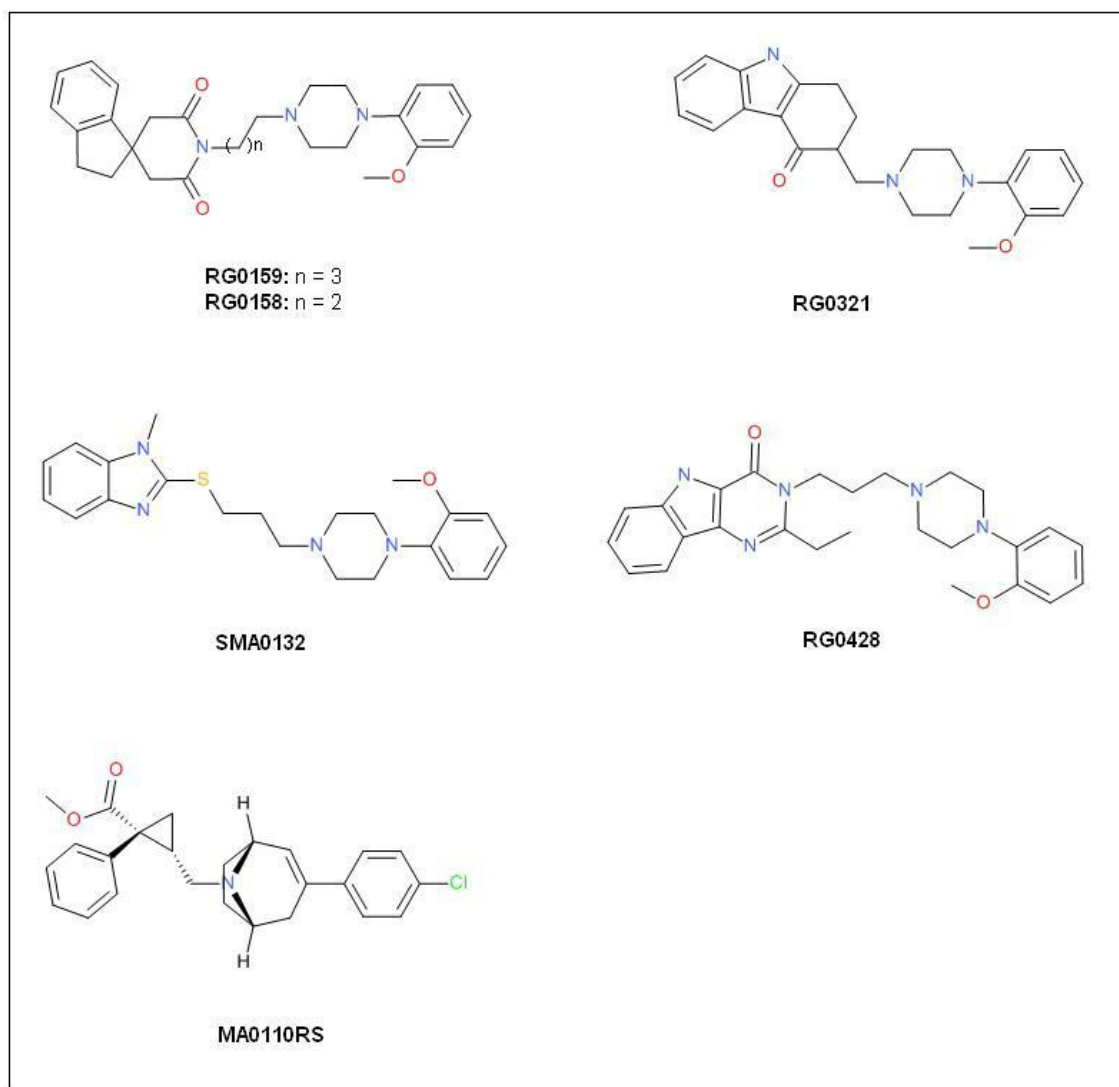


Fig. 2. Compounds under study that showed non-selective inhibition of Nav1.7 and Nav1.5.

4. CONCLUSION

Overall pharmaceutical companies, might take advantage of academic collections for hits fishing , with the advantage of increased chances to reduce costs as well share own research goals . Indeed the adhesion to Lilly OIDD program [4] allowed us to evaluate our molecules through cheminformatics tools in the Lilly OIDD web platform [6], which allowed to identify molecules to be candidates for the in vitro screening as well through phenotypic and target-based assays modules which represent interesting therapeutic areas [20].

In conclusion, a series of abandoned compounds have been rescued and repositioned for a new opportunity of research. In this perspective the above-mentioned compounds will be subject of further investigations concerning the structural properties as inhibitory ligands of Nav1.7 as well as structural relationship in the selectivity between Nav1.7 and Nav1.5.

Nowadays and still more in the future, approaches based on re-use and re-evaluation will be a necessity and also an opportunity in every context of pharmaceutical research.

REFERENCES

1. Ashburn, T. T.; Thor, K. B. Drug repositioning: identifying and developing new uses for existing drugs. *Nat Rev Drug Discov* 2004, 3, 673-683.
2. Dib-Hajj, S. D.; Yang, Y.; Black, J. A.; Waxman, S. G. The Nav1.7 sodium channel: from molecule to man. *Nat Rev Neurosci* 2013, 14, 49–62.
3. Horton, J.; Cohen, J.; Hobbs, H. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res* 2009, No. 50, 172-177.
4. openinnovation.lilly.com. <https://openinnovation.lilly.com/dd/> (accessed Jan 2015).
5. www.accelrys.com. <http://accelrys.com/resource-center/downloads/freeware/index.html> (accessed January 2015).
6. openinnovation.lilly.com. <https://openinnovation.lilly.com/dd/evaluating-compounds/in-silico-structure-selection.html> (accessed Jan 2015).
7. Bruns, R. F.; Watson, I. A. Rules for Identifying Potentially Reactive or Promiscuous Compounds. *J Med Chem* 2012, 55, 9763–9772.
8. Muegge, I. Selection criteria for drug-like compounds. *Med Res Rev* 2003, 23, 302–321.
9. Willett, P.; Barnard, J. M.; Downs, G. M. Chemical Similarity Searching. *J Chem Inf Comput Sci* 1998, 38, 983–996.
10. Eli Lilly & Company and the National Center for Advancing Translational Sciences. Assay Guidance Manual, 2004. www.ncbi.nlm.nih.gov.
Available: <http://www.ncbi.nlm.nih.gov/books/NBK53196/toc/> (accessed Jan 2015).
11. Pittalà, V.; Modica, M.; Salerno, L.; Siracusa, M.; Guerrera, F.; Mereghetti, I.; et al. Synthesis and endothelin receptor binding affinity of a novel class of 2-substituted-4-aryl-3-quinolinecarboxylic

acid derivatives. *Med Chem* 2008, 4 (2), 129-137.

12. Romeo, G.; Materia, L.; Marucci, G.; Modica, M.; Pittalà, V.; Salerno, L.; et al. New pyrimido[5,4-b]indoles and [1]benzothieno[3,2-d]pyrimidines: High affinity ligands for the α_1 -adrenoceptor subtypes. *Bioorg Med Chem Lett* 2006, 1 (6), 6200–6203.
13. Salerno, L.; Modica, M.; Romeo, G.; Pittalà, V.; Siracusa, M.; Amato, M.; et al. Novel inhibitors of nitric oxide synthase with antioxidant properties. *Eur J Med Chem* 2012, 49, 118-126.
14. Li, H.; Dong, B.; Park, S. W.; Lee, H. S.; Chen, W.; Liu, J. Hepatocyte Nuclear Factor 1 α Plays a Critical Role in PCSK9 Gene Transcription and Regulation by the Natural Hypocholesterolemic Compound Berberine. *J Biol Chem* 2009, 284, 28885-28895.
15. Romeo, G.; Materia, L.; Modica, M.; Pittalà, V.; Salerno, L.; Siracusa, M.; et al. Novel 4-phenylpiperidine-2,6-dione derivatives. Ligands for α_1 -adrenoceptor. *Eur J Med Chem* 2011, 46 (7), 2676-2690.
16. Romeo, G.; Materia, L.; Pittalà, V.; Modica, M.; Salerno, L.; Siracusa, M.; et al. New 1,2,3,9-tetrahydro-4H-carbazol-4-one derivatives: Analogues of HEAT as ligands for the α_1 -adrenergic receptor subtypes. *Bioorg Med Chem* 2006, 14, 5211–5219.
17. Romeo, G.; Materia, L.; Manetti, F.; Cagnotto, A.; Mennini, T.; Nicoletti, F.; et al. New Pyrimido[5,4-b]indoles as Ligands for α_1 -Adrenoceptor Subtypes. *J Med Chem* 2003, 46, 2877-2894.
18. Siracusa, M.; Salerno, L.; Modica, M.; Pittalà, V.; Romeo, G.; Amato, M.; et al. Synthesis of New Arylpiperazinylalkylthiobenzimidazole, Benzothiazole, or Benzoxazole Derivatives as Potent and Selective 5-HT_{1A} Serotonin Receptor Ligands. *J Med Chem* 2008, 51, 4529–4538.
19. Marrazzo, A.; Cobos, E. J.; Parenti, C.; Aricò, G.; Marrazzo, G.; Ronsisvalle, S.; et al. Novel Potent and Selective σ Ligands: Evaluation of Their Agonist and Antagonist Properties. *J Med Chem* 2011, 54, 3669-3673.
20. openinnovation.lilly.com. <https://openinnovation.lilly.com/dd/science-of-open-innovation/strategic-areas-of-interest.html> (accessed Jan 2015).

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