ACS Medicinal Chemistry Letters

Letter

GPBAR1 activation by C6-substituted hyodeoxycholane analogs protect against colitis

Simona De Marino, Claudia Finamore, Michele Biagioli, Adriana Carino, Silvia Marchianò, Rosalinda Roselli, Cristina Di Giorgio, Martina Bordoni, Francesco Saverio Di Leva, Ettore Novellino, Chiara Cassiano, Vittorio Limongelli, Angela Zampella, Carmen Festa, and Stefano Fiorucci

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.9b00636 • Publication Date (Web): 02 Mar 2020

Just Accepted

GPBAR1 activation by C6-substituted hyodeoxycholane analogs protect against colitis

Simona De Marino,[1] Claudia Finamore,[1] Michele Biagioli,[2] Adriana Carino,[2] Silvia Marchianò,[2] Rosalinda Roselli,[1] Cristina Di Giorgio,[2] Martina Bordoni,[2] Francesco Saverio Di Leva,[1] Ettore Novellino,[1] Chiara Cassiano,[1] Vittorio Limongelli,[1,3] Angela Zampella,[1] Carmen Festa[1,*] and Stefano Fiorucci.[2]

[1]Department of Pharmacy, University of Naples "Federico II", Via D. Montesano 49, 80131 Naples, Italy.

[2]Department of Surgery and Biomedical Sciences, Nuova Facoltà di Medicina, Perugia, Italy.

[3]Università della Svizzera italiana (USI), Faculty of Biomedical Sciences, Institute of Computational Science - Center for Computational Medicine in Cardiology, Via G. Buffi 13, CH-6900 Lugano, Switzerland.

KEYWORDS. GPBAR1 agonists, bile acid receptor, hyodeoxycholane derivatives, inflammatory bowel disease (IBD), colitis.

ABSTRACT: GPBAR1 agonists have been identified as potential lead for the treatment of diseases related to colon inflammation such as Crohn's and ulcerative colitis. In this paper, we report the discovery of a small library of hyodeoxycholane analogs, decorated at C-6 with different substituents, as potent and selective GPBAR1 agonists. *In vitro* pharmacological assays showed that compound **6** selectively activates GPBAR1 (EC_{50} = 0.3 μM) and reduces the production of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α,) in THP1 cells. The binding mode of compound **6** in GPBAR1 was elucidated by docking calculations. Moreover, compound **6** protects against TNBS-induced colitis in $Gpbar1^{+/+}$ rodent model representing an intriguing lead for the treatment of these inflammatory disorders.

In the last decades the onset of several chronic inflammatory disorders is increased in the worldwide population. Pathologies such as inflammatory type 2 diabetes mellitus, metabolic syndrome, inflammatory bowel disease (IBD), atherosclerosis and rheumatoid arthritis have become more and more frequent. Inflammatory bowel disease (IBD) is a common disorder that involves chronic remittent or progressive inflammatory conditions of gastrointestinal tract. Crohn's disease (CD), a life-long disease, and ulcerative colitis (UC), are the most common forms of IBD. Multiple factors play an important role in the pathogenesis of IBD. Among these may be included diet, immunological factors, environmental factors, microbiome, infectious agents and genetic susceptibility.[1]

The current treatment of IBD includes anti-inflammatory aminosalicylates and corticosteroids, antibiotics, biologic, immunosuppressive and anti-TNF-α agents.[2]

The lack of alternative approaches and the limited efficacy of this treatment, due to the incomplete remission and the arising of severe side effects, urge for new pharmacological tools, including molecules able to regulate the intestinal immune response. In this scenario, the targeting of GPBAR1 constitutes a therapeutic option for the treatment of IBDs.

GPBAR1, cell-surface G-protein coupled bile acid receptor 1 (also known as TGR5 or M-BAR1), belongs to the rhodopsin-like superfamily of G protein coupled receptors (GPCRs) and can be activated by a wide range of ligands, including endogenous BAs primarily lithocholic acid (LCA), deoxycholic acid (DCA) and their taurine-conjugates, tauro-LCA (TLCA) and tauro-DCA (TDCA) as well as many steroids.[3-6] Activation of GPBAR1 results in the increase of intracellular cAMP levels, which in turn triggers specific intracellular signalling cascades. In the human body, GPBAR1 is expressed in the gallbladder, small intestine, and is also located in tissues and cells not participating in the enterohepatic circulation such as muscle, brain, adipose tissue, immune system, and endothelial cells.[7]

Thus in recent years, academic and industrial investigations have been focused on the development of steroidal and non-steroidal GPBAR1 modulators useful in the treatment of enterohepatic and metabolic disorders.[8-12]

GPBAR1 has an important role in intestinal homeostasis and inflammation-driven immune disfunction. Previous studies have shown that $Gpbar1^{-/-}$ mice have an altered intestinal morphology with increased permeability and higher susceptibility to develop colitis. In addition, in the immune system, GPBAR1 reduces phagocytic activity and the production of pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6, and IL-8).[13]

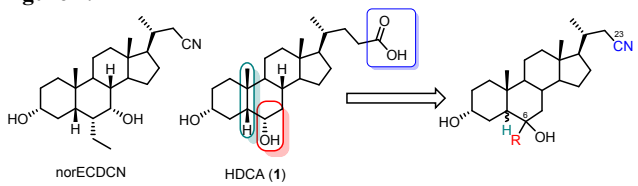
Recently, manipulating bile acid scaffolds, we succeeded in the discovery of potent and selective GPBAR1 agonists and demonstrated that GPBAR1 agonism shifts macrophagic population toward an anti-inflammatory M2 phenotype in mouse models of colitis, thus confirming that targeting GPBAR1 can be a valid therapeutic option in IBDs.[14]

In this context, our speculation started from the observations that the concomitant introduction of an alkyl group at C-6 and a nitrile end group on C-23 side chain produces a potent and

selective GPBAR1 agonist (NorECDCN in Figure 1),[15] and that hyodeoxycholic acid (HDCA, **1**),[16,17] a natural secondary bile acid, is a weak GPBAR1 agonist.[18,19] Thus, combining this information, and considering that chemical modifications on bile acid scaffold profoundly influence the pharmacokinetic properties and the activity towards BAs receptors,[20] our medicinal chemistry strategy has consisted in the evaluation of the effect on GPBAR1 activation, introducing a nitrile group in the side chain and different alkyl, alkenyl or aromatic substituents at C-6 on HDCA (Figure 1).

Since GPBAR1 can be activated by many natural steroids and terpenoids featuring a flat shape,[5] we have speculated on the stereochemistry of A/B ring junction of the steroidal nucleus in order to investigate the effect of physical shape change on the affinity towards GPBAR1.

Figure 1.



The synthetic protocol started with HDCA (**1**) that was first formylated at C-3 and C-6 hydroxyl groups and then subjected to one-carbon degradation at C-24 through the so-called second order "Beckmann rearrangement"[21]. Alkaline conditions (NaOH 6N) used in the treatment of the reaction afforded the C-3 and C-6 deprotection giving the *nor*nitrile (**2**) (Scheme 1). Oxidation with pyridinium dichromate (PDC) afforded a mixture of C-6 and C-3 mono and di-keto derivatives which were efficiently separated by flash chromatography to obtain the desired 6-keto *nor*nitrile (**3**) in 60% chemical yield. Treatment with several Grignard reagents gave 6-substituted compounds **4-14** showed in the Scheme 1.

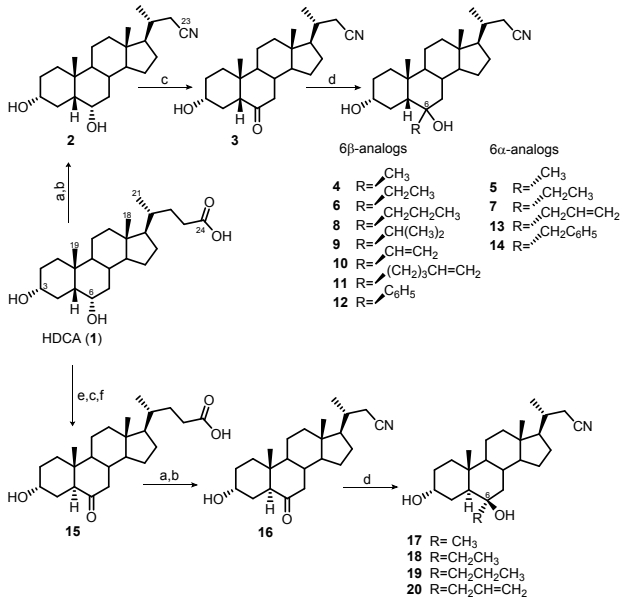
From a stereochemical point of view, treatment with methyl and ethyl magnesium bromides produced a mixture of 6α- and 6β-alkylated derivatives, in a 63/37 ratio for compounds **4** and **5** and in a 81/19 ratio for compounds **6** and **7**, efficiently separated by HPLC, where invariably the 6α-alkyl epimers (**5** and **7**) were eluted more rapidly than the corresponding 6β-alkyl ones (**4** and **6**).[22] Of interest, in agreement with the steric influence played by the ring A oriented on the α-face of ring B, the reaction with more hindered/longer Grignard reagents, such as propyl-, isopropyl-, vinyl-, 4-pentenyl-, phenyl- magnesium bromide, proceeded in a stereoselective manner, affording the exclusive formation of the corresponding 6β epimers **8-12**, through the approach of the Grignard reagent from the upper-face of the steroidal nucleus.

Finally, the treatment with allyl- and benzyl magnesium bromides afforded surprisingly the 6α-substituted compounds **13** and **14**.

Structural and stereochemical characterization was based on careful analysis of NMR data. According to literature data,[23] in the ^{1}H NMR spectra, the β-orientation of the hydroxyl group at C-6 as in compounds **5** and **7** produces the diagnostic down-field shifted signal for CH_3-19 (i.e. δ_H 1.14 in **5** *vs* 0.98 in **4**; δ_H 1.15 in **7** *vs* 1.01 in **6**). Additionally, NOESY cross peaks H_2-24/H-5, H-8 and H_3-19 confirmed the 6β-orientation of the ethyl group at C-6 on compound **6**.

To access to compounds **17-20** with the *trans* A/B ring junction, HDCA (**1**) was subjected to methylation on the side chain and then to C-6 regioselective oxidation as previously described. Alkaline hydrolysis at the side chain methyl ester produced the concomitant epimerization at C-5 affording compound **15** in quantitative yield. One-carbon degradation at C-24 followed by Grignard reactions, as previously described, afforded compounds **17-20**. In agreement with the flat shape due to the *trans* A/B ring junction and the steric influence played by the methyl group at C-10, the approach of the nucleophile proceeded from the α face of the steroidal nucleus producing exclusively the 6α-alkyl epimers (Scheme 1).

Scheme 1. Synthetic procedure to prepare derivatives 4-14 and 17-20[a]



[a]*Reagents and conditions*. a) HCOOH, $HClO_4$, 45-50°C; b) TFA, trifluoroacetic anhydride, $NaNO_2$, 45-50°C and then NaOH, 90% over two steps; c) PDC, in DCM dry, 60% yield; d) Methyl-, ethyl-, propyl-, isopropyl-, allyl-, vinyl-, 4-pentenyl-, phenyl- and benzyl-magnesium bromides, in THF dry, 0°C; e) p-TsOH, MeOH dry; f) NaOH, MeOH:H_2O (1:1), reflux, quantitative yield.

The synthetized compounds were evaluated on GPBAR1 in transactivation assays. They were also tested on Farnesoid X Receptor (FXR) in order to determine their activity towards this related nuclear bile acid receptor. For this purpose, Hek293T cells transfected with GPBAR1 and HepG2 cells transfected with FXR, were incubated with 10 μM of compounds **2** and **4-20**. Activities of compounds on GPBAR1 and FXR were compared to those of the reference compounds TLCA and CDCA, respectively, that were set as 100% of activity. As shown in Table 1, all tested compounds, with a *cis* A/B ring junction, were effective in transactivating GPBAR1, while they exerted a minimal effect on FXR, showing a marked selectivity toward GPBAR1 over this receptor (Table 1). Compounds **17-20**, endowed with a *trans* junction, showed no agonistic activity towards GPBAR1 and FXR (Figures S2 and S3).

Results shown in Table 1 demonstrated that the best match in terms of % efficacy, EC_{50} and selectivity has been found for compounds **4-7** and **11**.

The physicochemical parameters of the most efficacious compounds were measured by LC-MS analysis. As shown in Table S1 all compounds exhibited good aqueous solubility and a suitable logD.

Therefore, we have investigated whether these compounds could exert an *in vitro* activity on GPBAR1-target genes. For this purpose, THP1 monocytic/macrophage cells, were primed with LPS (lipopolysaccharide), and co-incubated with or

without compounds **4-7** and **11** at 10 μM. As shown in Figure 2, all compounds reduced the production of pro-inflammatory cytokines (IL-1β, IL-6) in THP1 cells but only compounds **4** and **6-7** decrease also the production of TNF-α.

Table 1. Efficacy, EC_{50} values and selectivity for compounds **2** and **4-14**

Compound	C-6 substituents	GPBAR1* Eff. (% vs TLCA)	FXR** Eff. (% vs CDCA)	GPBAR1# EC_{50} μM	GPBAR1/FXR## Selectivity
2	H	118.7 ± 3.16	3.5 ± 0.34	2.65	33.9
4	6β-CH_3	95.4 ± 0.24	6.4 ± 0.23	6.9	14.9
5	6α-CH_3	83.4 ± 2.4	5.5 ± 0.7	0.57	15.1
6	6β-CH_2CH_3	101.2 ± 4.1	12.3 ± 0.47	0.3	8.2
7	6α-CH_2CH_3	65.9 ± 1.69	6.6 ± 0.065	0.66	9.9
8	6β-$CH_2CH_2CH_3$	67.1 ± 2.1	3.1 ± 0.29	1.2	21.6
9	6β-$CH(CH_3)_2$	22.2 ± 0.13	4.3 ± 0.31	2.78	5.16
10	6β-$CH{=}CH_2$	62.5 ± 2.18	2.2 ± 0.5	2.26	28.4
11	6β-$(CH_2)_3CH{=}CH_2$	90.1 ± 1.35	1.6 ± 0.05	0.78	56.31
12	6β-C_6H_5	38.8 ± 1.79	18.2 ± 1.08	4.58	2.13
13	6α-$CH_2CH{=}CH_2$	62.5 ± 2.15	4.1 ± 0.04	0.1	15.2
14	6α-$CH_2C_6H_5$	8.3 ± 0.015	4.4 ± 0.49	9.33	1.88

*Activity toward GPBAR1 in a reporter assay was assessed in HEK-293T cells transfected with a cAMP responsive element (CRE) cloned upstream to the luciferase gene. For calculation of efficacy data, maximal transactivation of CRE caused by each compound (10 μM) was compared to maximal transactivation caused by TLCA (10 μM). **Activity toward FXR in a reporter assay was assessed in HepG2 cells transfected with a FXR responsive element (IR1) cloned upstream to the luciferase gene. For calculation of efficacy data, maximal transactivation of IR1 caused by each compound (10 μM) was compared to maximal transactivation caused by CDCA (10 μM). #GPBAR1 affinity expressed as EC_{50} values. ##GPBAR1/FXR selectivity calculated as ratio between % of activity toward GPBAR1 and % of activity toward FXR. Data are calculated from the same experiment conducted in triplicate.

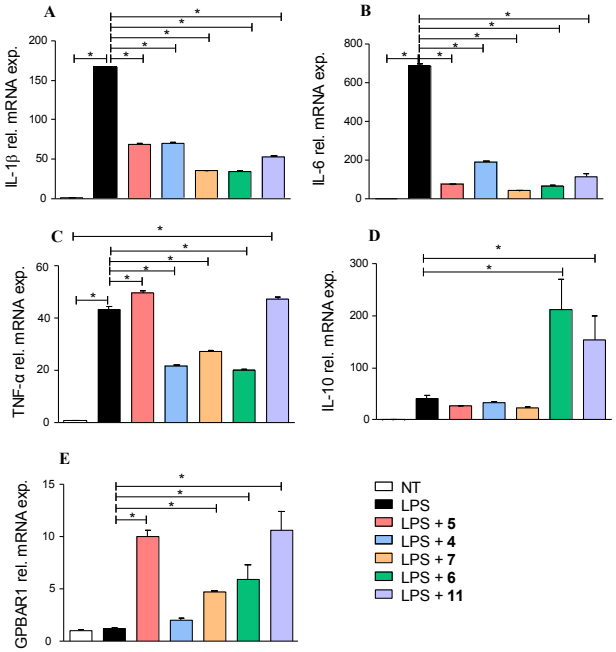


Figure 2. *In vitro* effects of selected compounds on human monocytes activation. Relative mRNA expression of pro- and anti-inflammatory genes was assessed by RT-PCR in THP1 cells: A) IL-1β; B) IL-6; C) TNF-α; D) IL-10 and E) GPBAR1, relative mRNA expression. Values are normalized relative to GAPDH mRNA and are expressed as mean ± SEM (n = 3); *p < 0.05.

Additionally, exposure to compound **6** increased the expression for anti-inflammatory gene IL-10, that is a specific target of GPBAR1.These results prompted us to investigate the effect of compound **6** in a TNBS-induced colitis model in Gpbar1$^{+/+}$ and Gpbar1$^{-/-}$ mice. The development and the severity of colitis in Gpbar1$^{+/+}$ mice, assessed by body weight loss, CDAI (Clinical Disease Activity Index) and histological sections, were attenuated by treatment with **6**. Conversely, this compound did not produce effects in the Gpbar1$^{-/-}$ mice (Figures 3 A-C).

We also analyzed the expression of pro- and anti-inflammatory cytokines in the colon of these animals (Figure 4). As expected, TNBS administration increased the expression of pro-inflammatory cytokines IL-6, IL-1β and TNF-α both in wild-type mice and in Gpbar1$^{-/-}$ mice. On the contrary, compound **6** was able to revert this inflammatory pattern by reducing the expression of pro-inflammatory cytokines and also increasing the expression of the anti-inflammatory cytokine, IL-10. These beneficial effects were lost in mice lacking the receptor.

To elucidate the binding mode of the newly developed HDCA analogues, we performed docking calculations that are widely used to generate and assess ligand/protein complexes based on scoring functions.[24-26] In particular, we investigated the most promising compound of the series, **6**, into the homology model of hGPBAR1 that we have previously reported and validated through drug design studies.[17,27,28]

In the best scored and most recurrent docking pose, compound **6** is predicted to bind GPBAR1 similar to other BA derivatives previously reported as agonists of this receptor.[17,27-29] The ligand's steroidal scaffold is hosted in a wide hydrophobic pocket where it can form favourable interactions with the side

chains of residues such as Leu71, Phe96, Leu174, and Trp237 (Figure 5). Additional Van der Waals contacts are formed by the 6β-ethyl group of **6** which occupies the ancillary pocket shaped by residues Leu97, Phe138 and Leu173.

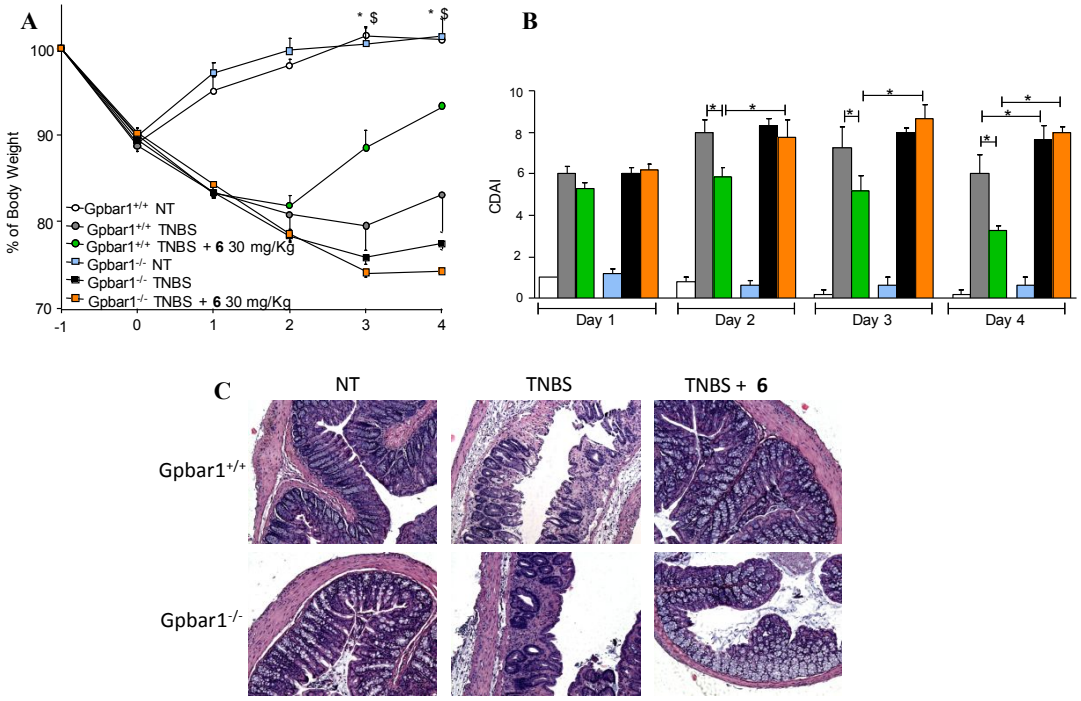


Figure 3. Compound **6** protects against colitis in a GPBAR1-dependent manner. Gpbar1$^{+/+}$ and Gpbar1$^{-/-}$ mice were treated with TNBS alone or in combination with compound **6** (30 mg/kg). A) Changes in body weight. B) CDAI of mice during the course of TNBS-induced colitis. C) H&E staining of colon sections from control mice, mice treated with TNBS, and mice treated with TNBS and compound **6** (original magnification 10x). (n = 5);$^{\#}p < 0.05$

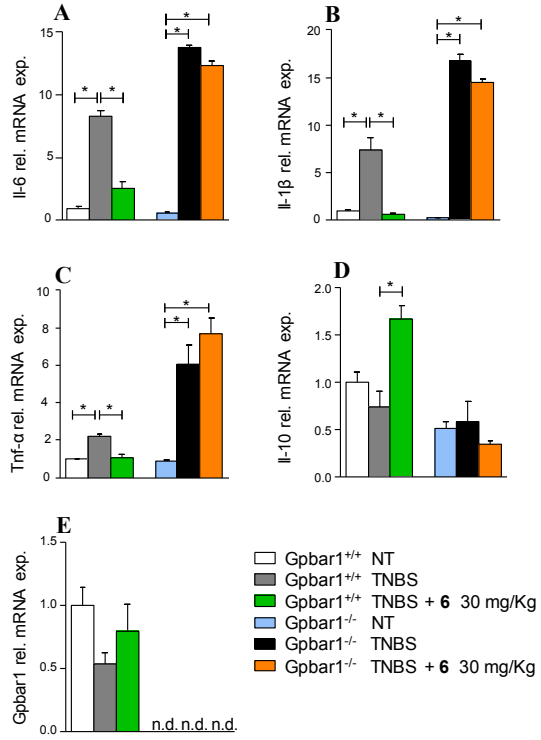


Figure 4. Compound **6** reversed TNBS-induced inflammation in a GPBAR1-dependent manner. Gpbar1$^{+/+}$ and Gpbar1$^{-/-}$ mice were treated with TNBS alone or in combination with compound **6** (30 mg/kg). Relative mRNA expression of pro- and anti-inflammatory genes was assessed by RT-PCR colon samples: A) Il-1β, B) Il-6, C) Tnf-α, D) Il-10 and E) Gpbar1, relative mRNA expression. Values are normalized relative to Gapdh mRNA and are expressed as mean ± SEM (n = 5); *$p < 0.05$.

Besides this hydrophobic interaction network, the 3α- and 6α-OHs of the ligand can establish H-bonds with the side chains of Glu169 on TM5 and Asn93 on TM3, respectively. On the opposite side of the binding cavity, the ligand side chain points toward the amphipathic cleft defined by the side chains of residues of TM helices 1, 2, and 7, which have been described in previous papers as interacting points for BAs derivatives.[27] Here, the ligand's nitrile group can form direct or water-mediated H-bond interactions with Ser267 and Ser270, which might contribute to stabilize the ligand binding pose.

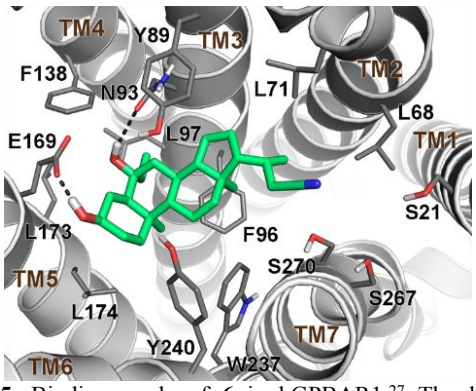


Figure 5. Binding mode of **6** in hGPBAR1.[27] The ligand is represented as green sticks, while the receptor is shown as gray cartoons. Amino acids involved in the ligand binding are depicted as sticks. Non-polar hydrogens are omitted for clarity, while H-bonds are shown as black dashed lines.

We note that the ligand interaction with Glu169 has been previously identified by us and by other authors as a major anchor point for BAs to GPBAR1, presumably triggering the receptor activation.[27,30]

On the other hand, the interaction with Asn93 was reported to play a minor role in the binding of BAs to GPBAR1. In fact, LCA derivatives display good GPBAR1 agonist profiles, although they do not present alcohol groups at either the C-6 or C-7 position that could interact with Asn93.[28] These data might explain our findings indicating that compound **7**, which displays an inverted configuration at C-6 and thus cannot interact with Asn93 (Figure S1), shows however a good EC_{50} value, comparable to that of **6** (Table 1).

In conclusion, we report the discovery of a new chemotype of GPBAR1 agonists designed on hyodeoxycholic acid scaffold. We have decorated the C-6 position with different substituents and speculated also on the stereochemistry of A/B ring junction of the steroidal nucleus obtaining a small library of derivatives. Interestingly, we note that compounds featured with a *cis* A/B ring junction showed a marked selectivity toward GPBAR1 over FXR in transactivation assays, exerting minimal activating effects on the latter receptor. Our studies identified compound **6** as potent and selective GPBAR1 agonist. The activation of the receptor, combined with the ability to revert both the expression of inflammatory genes *in vitro* and the inflammatory pattern in TNBS-induced colitis model in a GPBAR1-dependent manner, indicate compound **6** as a promising lead for the treatment of GPBAR1-related inflammatory bowel disorders.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures; binding mode of compound **7** in hGPBAR1; transactivation assay on GPBAR1 of compounds **17-20**; AlphaScreen assay on FXR of compounds **17-20**; transactivation assays on LXRα, LXRβ, and PPARγ of compound **6**; Physiochemical properties of compounds **4-7** and **11**; ^{1}H NMR of compounds **2**, **4-14** and **17-20**; NOESY spectrum of compound **6** and ROESY spectrum of compound **8** (PDF).

AUTHOR INFORMATION

Corresponding Author

*Dr. Carmen Festa; e-mail: carmen.festa@unina.it

Notes

Stefano Fiorucci and Angela Zampella have filed the Italian patent application No. 102019000023403 in the name of PRECISION BIO-THERAPEUTICS S.R.L., a spinoff of the University of Perugia, on same the compounds described in this paper.

ABREVIATIONS

BA, Bile acid; CDAI, colitis disease activity index; CDCA, chenodeoxycholic acid; DCM, dichloromethane; FXR, farnesoid X receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPBAR1, G protein-coupled bile acid receptor 1; HDCA, hyodeoxycholic acid; IBD, inflammatory bowel disease; IL-6, interleukin 6; IL-1β, interleukin 1β; IL-10, interleukin 10; LCA, lithocholic acid; LDB, ligand binding domain; LPS, lipopolysaccharide; NOESY, Nuclear Overhauser Effect SpectroscopY; PDC, pyridinium dichromate; TNBS, 2,4,6-Trinitrobenzenesulfonic acid; TNF-α, Tumor necrosis factor alpha; TFA, trifluoroacetic acid; TLCA, taurolithocholic acid.

REFERENCES

(1) Knight-Sepulveda, K.; Kais, S.; Santaolalla, R.; Abreu, M. T. Diet and inflammatory bowel disease. *Gastroenterol. Hepatol.* **2015**, *11*, 511-520.

(2) Mosli, M. H.; Rivera-Nieves, J.; Feagan. B. G. T-cell trafficking and anti-adhesion strategies in inflammatory bowel disease: current and future prospects. *Drugs* **2014**, *74*, 297-311.

(3) Maruyama, T.; Miyamoto, Y.; Nakamura, T.; Tamai, Y.; Okada, H.; Sugiyama, E.; Nakamura, T.; Itadani, H.; Tanaka, K. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem. Biophys. Res. Commun.* **2002**, 298, 714-719.

(4) Watanabe, M.; Houten, S. M.; Mataki, C.; Christoffolete, M. A.; Kim, B. W.; Sato, H.; Messaddeq, N.; Harney, J. W.; Ezaki, O.; Kodama, T.; Schoonjans, K.; Bianco, A. C.; Auwerx, J. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* **2006**, *439*, 484-489.

(5) De Marino, S., Festa, C., Sepe, V., Zampella, A. Chemistry and pharmacology of GPBAR1 and FXR selective agonists, dual agonists, and antagonists. *Handb. Exp. Pharmacol*. **2019**, *256*, 137-165.

(6) Imperatore, C.; D'Aniello, F.; Aiello, A.; Fiorucci, S.; D'Amore, C.; Sepe, V.; Menna, M. Phallusiasterols A and B: Two new sulfated sterols from the mediterranean tunicate *Phallusia fumigata* and their effects as modulators of the PXR receptor. *Mar. Drugs* **2014**, *12*, 2066–2078.

(7) Perino, A.; Schoonjans, K. TGR5 and immunometabolism: Insights from physiology and pharmacology. *Trends Pharmacol. Sci.* **2015**, *36*, 847-57.

(8) Gioiello, A.; Rosatelli, E.; Nuti, R.; Macchiarulo, A.; Pellicciari, R. Patented TGR5 modulators: a review (2006 -- present). *Expert Opin. Ther. Pat*. **2012**, 22,1399-414.

(9) Xu, Y. Recent progress on bile acid receptor modulators for treatment of metabolic diseases. *J. Med. Chem*. **2016**, *59*, 6553-6579.

(10) Duan, H.; Ning, M.; Zou, Q.; Ye, Y.; Feng, Y.; Zhang, L.; Leng, Y.; Shen, J. Discovery of intestinal targeted TGR5 agonists for the treatment of type 2 diabetes. *J. Med. Chem.* **2015**, *58*, 8, 3315-3328.

(11) Chen, T.; Reich, N. W.; Bel, N.; Finn, P. D.; Rodriguez, D.; Kohler, J.; Kozuka, K.; He, L.; Spencer, A. G.; Charmot, D.; Navre, M.; Carreras, C. W.; Koo-McCoy, S.; Tabora, J.; Caldwell, J. S.; Jacobs, J. W.; Lewis, J. G. Design of gut-restricted thiazolidine agonists of G Protein-Coupled Bile Acid Receptor 1 (GPBAR1, TGR5). *J. Med. Chem.* **2018**, *61*, 7589-7613.

(12) Cao, H.; Chen, Z. X.; Wang,K.; Ning, M. M.; Zou, Q. A.; Feng, Y.; Ye, Y. L.; Leng, Y.; Shen, J. H. Intestinally-targeted TGR5 agonists equipped with quaternary ammonium have an improved hypoglycemic effect and reduced gallbladder filling effect. *Sci. Rep.* **2016**, *6*, 28676.

(13) Cipriani, S.; Mencarelli, A.; Chini, M. G.; Distrutti, E.; Renga, B.; Bifulco, G.; Baldelli, F.; Donini, A.; Fiorucci, S. The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis. *Plos One* **2011**, *6*, e25637.

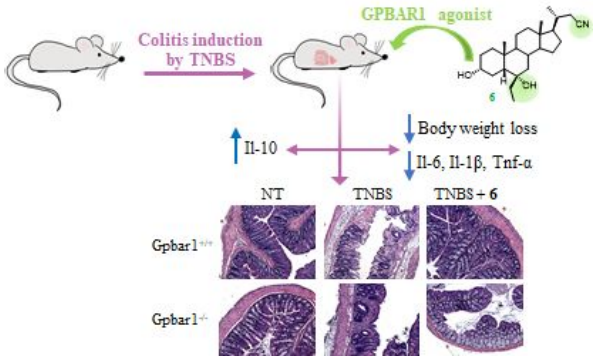
(14) Biagioli, M.; Carino, A.; Cipriani, S.; Francisci, D.; Marchianò, S.; Scarpelli, P.; Sorcini, D.; Zampella, A.; Fiorucci, S. The bile acid receptor GPBAR1 regulates the M1/M2 phenotype of intestinal macrophages and activation of GPBAR1 rescues mice from murine colitis. *J. Immunol*. **2017**, *199*, 718-733.

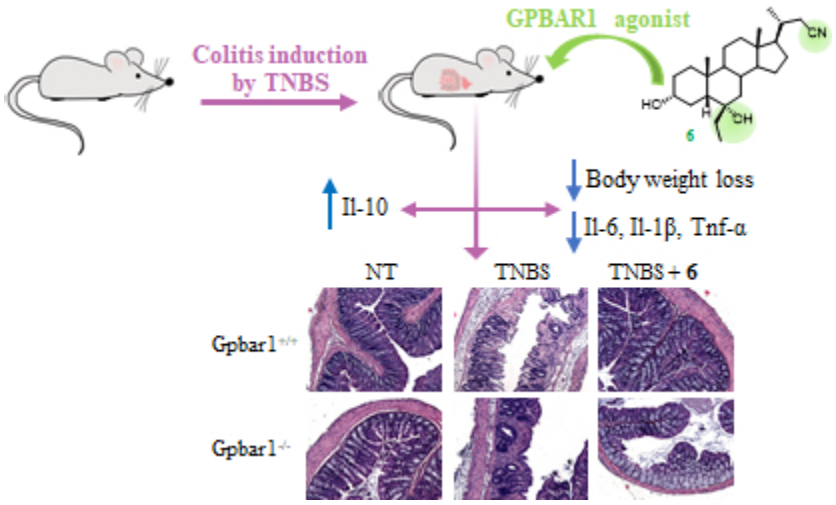
(15) Festa, C.; Renga, B.; D'Amore, C.; Sepe, V.; Finamore C.; De Marino, S.; Carino, A.; Cipriani, S.; Monti, M. C.; Zampella, A.; Fiorucci,S. Exploitation of cholane scaffold for the discovery of potent and selective farnesoid X receptor (FXR) and G-protein coupled bile acid receptor 1 (GP-BAR1) ligands. *J. Med. Chem.*, **2014**, *57*, 8477-8495.

(16) De Marino, S.; Carino, A.; Masullo, D.; Finamore, C.; Sepe, V.; Marchianò, S.; Di Leva, F. S.; Limongelli, V.; Fiorucci, S.; Zampella, A. Epoxide functionalization on cholane side chains in the identification of G-protein coupled bile acid receptor (GPBAR1) selective agonists. *RSC Adv*. **2017**, *7*, 32877-32885.

(17) De Marino, S.; Carino, A.; Masullo, D.; Finamore, C.; Marchianò, S.; Cipriani, S.; Di Leva, F. S.; Novellino, E.; Catalanotti, B.; Limongelli, V.; Fiorucci, S.; Zampella, A. Hyodeoxycholic acid derivatives as liver X receptor α and G-protein-coupled bile acid receptor agonists. *Sci. Rep*. **2017**, 7:43290.

(18) Song, C.; Hiipakka, R. A.; Liao, S. Selective activation of liver X receptor alpha by 6-alpha-hydroxy bile acids and analogs. *Steroids* **2000**, *65*, 423-427.
(19) Sato, H.; Macchiarulo, A.; Thomas, C.; Gioiello, A.; Une, M.; Hofmann, A. F.; Saladin, R.; Schoonjans, K.; Pellicciari, R.; Auwerx J. Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies. *J. Med. Chem*. **2008**, *51*, 1831-1841.
(20) Roda, A.; Pellicciari, R.; Gioiello, A.; Neri, F.; Camborata, C.; Passeri, D.; De Franco, F.; Spinozzi, S.; Colliva, C.; Adorini, L.; Montagnani, M.; Aldini, R. Semisynthetic bile acid FXR and TGR5 agonists: Physicochemical properties, pharmacokinetics, and metabolism in the rats. *J. Pharmacol. Exp. Ther*. **2014**, *350*, 56-68.
(21) Schteingart, C. D.; Hofmann, A. F. Synthesis of 24-nor-5β-cholan-23-oic acid derivatives: a convenient and efficient one-carbon degradation of the side chain of natural bile acids. *J. Lipid Res.* **1988**, *29*, 1387-1395.
(22) Une, M.; Cohen, B. I.; Mosbach, E. H. New bile acid analogs: 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid, 3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid, and 3α-hydroxy-7ξ-methyl-5β-cholanoic acid. *J. Lipid Research* **1984**, *25*, 407-410.
(23) Matoba, N.; Mosbach, E. H.; Cohen, B. I.; Une, M.; McSherry, C. K. Synthesis of new bile acid analogues and their metabolism in the hamster: *3α,*6α-dihydroxy-6βmethyl-5β-cholanoic acid and 3α,6β-dihydroxy-6α-methyl-5β-cholanoic acid, *J. Lipid Res*. **1989**, *30*, 1005-1014.
(24) Anzini, M.; Braile, C.; Valenti, S.; Cappelli, A.; Vomero, S.; Marinelli, L.; Limongelli, V.; Novellino, E.; Betti, L.; Giannaccini, G.; Lucacchini, A.; Ghelardini, C.; Norcini, M.; Makovec, F.; Giorgi, G.; Ian Fryer, R. Ethyl 8-fluoro-6-(3-nitrophenyl)-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate as novel, highly potent, and safe antianxiety agent. *J. Med. Chem.* **2008**, *51*, 4730-4743.
(25) Famiglini, V.; La Regina, G.; Coluccia, A.; Pelliccia, S.; Brancale, A.; Maga, G.; Crespan, E.; Badia, R.; Riveira-Muñoz, E.; Esté, J.A.; Ferretti, R.; Cirilli, R.; Zamperini, C.; Botta, M.; Schols, D.; Limongelli, V.; Agostino, B.; Novellino, E.; Silvestri, R. Indolylaryl-sulfones carrying a heterocyclic tail as very potent and broad spectrum HIV-1 non-nucleoside reverse transcriptase inhibitors. *J. Med. Chem.* **2014**, *57*, 9945-9957.
(26) Nuti, E.; Casalini, F.; Avramova, S.I.; Santamaria, S.; Fabbi, M.; Ferrini, S.; Marinelli, L.; La Pietra, V.; Limongelli, V.; Novellino, E.; Cercignani, G.; Orlandini, E.; Nencetti, S.; Rossello, A. Potent Arylsulfonamide Inhibitors of Tumor Necrosis Factor-α Converting Enzyme Able to Reduce Activated Leukocyte Cell Adhesion Molecule Shedding in Cancer Cell Models. *J. Med. Chem.* **2010**, *53*, 2622-2635.
(27) D'Amore, C.; Di Leva, F. S.; Sepe, V.; Renga, B.; Del Gaudio, C.; D'Auria, M. V.; Zampella, A.; Fiorucci, S.; Limongelli, V. Design, synthesis, and biological evaluation of potent dual agonists of nuclear and membrane bile acid receptors. *J. Med. Chem.* **2014**, *57*, 937-954.
(28) Di Leva, F. S.; Festa, C.; Renga, B.; Sepe, V.; Novellino, E.; Fiorucci, S.; Zampella, A.; Limongelli, V. Structure-based drug design targeting the cell membrane receptor GPBAR1: exploiting the bile acid scaffold towards selective agonism. *Sci. Rep*. **2015**, *5*, 16650.
(29) Di Leva, F. S.; Di Marino, D.; Limongelli, V. Structural insight into the binding mode of FXR and GPBAR1 modulators. *Handb. Exp. Pharmacol.* **2019**, *256*, 111-136.
(30) Gertzen C. G. W.; Spomer, L.; Smits, S. H. J.; Häussinger, D.; Keitel, V.; Gohlke, H. Mutational mapping of the transmembrane binding site of the G-protein coupled receptor TGR5 and binding mode prediction of TGR5 agonists. *Eur. J. Med. Chem*. **2015,** *104*, 57-72.


Colitis induction by TNBS
GPBAR1 agonist
Il-10
Body weight loss
Il-6, Il-1β, Tnf-α
NT
TNBS
TNBS + 6
Gpbar1+/+
Gpbar1-/-



35x21mm (300 x 300 DPI)