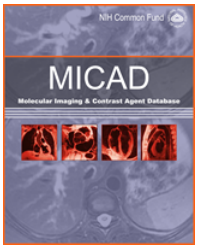
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Cheng KT, Smith CJ, Ma L, et al. Alexa Fluor 680-glycylglycylglycine-bombesin[7-14]NH_2 peptide. 2007 Jul 20 [Updated 2007 Aug 25]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



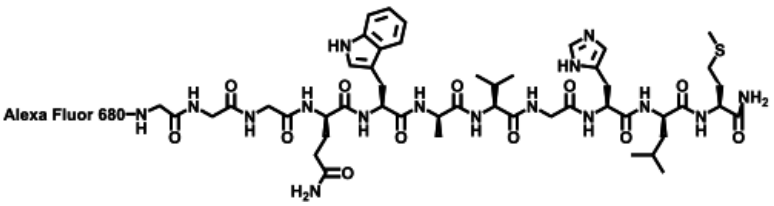
Alexa Fluor 680-glycylglycylglycine-bombesin[7-14]NH_2 peptide

Alexa Fluor 680-G-G-G-BN[7-14]NH_2

Kenneth T. Cheng, PhD,[1] Charles J. Smith, PhD,[2] Lixin Ma, PhD,[3] Ping Yu, PhD,[4] and Timothy J. Hoffman, PhD[5]

Created: July 20, 2007; Updated: August 25, 2007.

Chemical name:	Alexa Fluor 680-glycylglycylglycine-bombesin[7-14]NH_2 peptide
Abbreviated name:	Alexa Fluor 680-G-G-G-BN[7-14]NH_2
Synonym:	Alexa Fluor 680-G-G-G-BBN[7-14]NH_2,, Alexa Fluor 680-BBN, Alexa Fluor 680-BN
Agent Category:	Peptide
Target:	Gastrin-releasing peptide receptor (GRP-R)
Target Category:	Receptor binding
Method of detection:	Optical (NIR fluorescence) imaging
Source of signal:	Alexa Fluor 680
Activation:	No



Author Affiliations: 1 National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov. 2 University of Missouri-Columbia, Columbia, MO, Corresponding Author; Email: smithcj@health.missouri.edu. 3 University of Missouri-Columbia, Columbia, MO; Email: mal@health.missouri.edu. 4 University of Missouri-Columbia, Columbia, MO; Email: yuping@missouri.edu. 5 University of Missouri-Columbia, Columbia, MO; Email: HoffmanT@health.missouri.edu.

Table continued from previous page.

Studies:	• *In vitro* • Rodents	Alexa Fluor 680-G-G-G-BN[7-14]NH_2 Click on protein, nucleotide (RefSeq), and gene for more information about BN and GRP.

Background

[PubMed]

Alexa Fluor 680-glycylglycylglycine-bombesin[7-14]NH_2 peptide (Alexa Fluor 680-G-G-G-BN[7-14]NH_2), a peptide analog of human gastrin-releasing peptide (GRP) conjugated with Alexa Fluor 680, was developed for optical imaging of tumors with overexpressed GRP receptors (1). Alexa Fluor 680 is a fluorescence dye with an absorption maximum of 679 nm, an emission maximum of 720 nm, and an extinction coefficient of 180,000 $cm^{-1}M^{-1}$ (2).

The amphibian bombesin (BBN or BN), a peptide of 14 amino acids, is an analog of human GRP (a peptide of 27 amino acids) that binds to GRP receptors (GRP-R) with high affinity and specificity (1, 3). Both GRP and BN share an amidated C-terminus sequence homology of 7 amino acids, -Trp-Ala-Val-Gly-His-Leu-Met-NH_2. BN-Like peptides have been shown to induce various biological responses in diverse tissues, including the central nervous system (CNS) and the gastrointestinal (GI) system (4, 5). They also act as potential growth factors for both normal and neoplastic tissues. Specific BN receptors (BN-R) have been identified on CNS and GI tissues and a number of tumor cell lines. The BN-R superfamily includes at least 4 different subtypes, namely the GRP-R subtype (BB2), the neuromedin B (NMB) receptor subtype (BB1), the BB3 subtype, and the BB4 subtype (6). The findings of GRP-R overexpression in various human tumors, such as breast, prostate, lung, colon, ovarian, and pancreatic cancers, provide opportunities for tumor imaging by designing specific molecular imaging agents to target the GRP-R (1, 7-10).

A review by Smith et al. (10) indicated varying degrees of success in the current development of GRP-R–targeted radiopharmaceuticals for diagnostic or therapeutic applications. Various BN analogs have been labeled with ^{99m}Tc and ^{111}In for single-photon emission computed tomography imaging. BN analogs labeled with ^{68}Ga-, ^{18}F-, or ^{64}Cu have been studied for positron emission tomography imaging (8, 11, 12). Optical imaging is a method that utilizes light photons emitted from bioluminescence and fluorescence probes (13). Depth penetration is a major limiting factor for *in vivo* optical imaging. Currently, *in vivo* optical imaging has wide applications in small animal imaging but only limited applications in large animal and human studies (14). Near-infrared (NIR) fluorescence imaging (light range, 650–900 nm) has the advantages of relatively higher tissue penetration and lower autofluorescence from nontarget tissue. Alexa fluorochromes are spectrally unique fluorescent probes with relatively high quantum yields in their excitation and emission wavelength ranges (2, 15). These fluorochromes can be used in succinimidyl ester form for conjugation with the primary amines of biomolecules. Ma et al. (1) prepared and evaluated the fluorescence probe Alexa Fluor 680-G-G-G-BN[7-14]NH_2 for optical imaging of breast cancer in mice.

Synthesis

[PubMed]

Ma et al. (1) reported the preparation of Alexa Fluor 680-G-G-G-BN[7-14]NH_2. The BN peptide has the sequence of H_2N-Gly-Gly-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (H_2N-G-G-G-Q-W-A-V-G-H-L-M-(NH_2)) and was synthesized by the standard 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry with use of an automated peptide synthesizer (16). In this solid-phase peptide synthesis, the reaction of *O*-benzotriazolyl-

tetramethyluronium hexafluorophosphate activated carboxyl groups on the reactant with the N-terminal amino group on the growing peptide provided for stepwise amino acid addition. Rink amide *O*-benzotriazolyl-tetramethyluronium hexafluorophosphate resin and the Fmoc-protected amino acids with side-chain protections were used to synthesize the NH_2-G-G-G-BN[7-14]NH_2 peptide. The final product was cleaved by a cocktail of trifluoroacetic acid, thioanisol, ethanedithiol, and water. The yield of the crude peptide was ~80%, which was purified by high-performance liquid chromatography (HPLC). The purified peptide was prepared as a 10-mg/ml solution with sodium bicarbonate (pH 8.5) for conjugation with the fluorescence label. The Alexa Fluor 680 succinimidyl ester in dimethylformamide was added to the NH_2-G-G-G-BN[7-14]NH_2 solution, and the reaction mixture was allowed to incubate at room temperature for 5 h in darkness. The resulting Alexa Fluor 680-G-G-G-BN[7-14]NH_2 conjugate was purified by HPLC. The yield of the peptide conjugate was ~60%. Coupling to the less reactive amidated C-terminus was not observed. Electrospray ionization mass spectrometry analysis showed a molecular ion of 1953.2, which was consistent with the calculated value of 1952.8.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Ma et al. (1) conducted *in vitro* competitive binding assays of Alexa Fluor 680-G-G-G-BBN[7-14]NH_2 for the GRP-R in human T-47D breast cancer cell line, which is known to express the GRP-R in very high numbers. Ma et al. used ^{125}I-Tyr^4-BN as the competitive ligand in these assays. The 50% inhibitory concentration (IC_{50}) of Alexa Fluor 680-G-G-G-BBN[7-14]NH_2 was determined to be 7.7 ± 1.4 nM. The efficiency of *in vitro* cell internalization and residualization of Alexa Fluor 680-G-G-G-BN[7-14]NH_2 was also studied in the T-47D cells (1). Cell washing and confocal fluorescence microscopy image analysis after 40 min of incubation showed that Alexa Fluor 680-G-G-G-BN[7-14]NH_2 was effectively internalized and retained within the cells. The study did not attempt to quantify the data. When 5 µg of wild-type BN[1-14] was incubated with the cells before incubation with Alexa Fluor 680-G-G-G-BN[7-14]NH_2, the binding and internalization of Alexa Fluor 680-G-G-G-BN[7-14]NH_2 were completely or significantly blocked.

Animal Studies

Rodents

[PubMed]

In vivo tumor localization studies of Alexa Fluor 680-G-G-G-BN[7-14]NH_2 were performed in severely compromised immunodeficient mice bearing T-47D human breast tumors on the left and right flanks. Each mouse received 25 nmol (50 µg) of Alexa Fluor 680-G-G-G-BN[7-14]NH_2 by i.v. administration. Fluorescence imaging was performed 15 min after administration in euthanized animals (n = 4). The images showed a high degree of tumor localization of the fluorescence label. The tumor location in the animal was confirmed by magnetic resonance imaging. Some degree of activity was observed in the collateral tissue of the abdomen. When 50 µg of BN[1-14] was administered to the mice before the injection of Alexa Fluor 680-G-G-G-BN[7-14]NH_2, little or no fluorescence signal was detected in the tumor tissue. The study did not attempt to quantify the data.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

References

1. Ma L., Yu P., Veerendra B., Rold T.L., Retzloff L., Prasanphanich A., Sieckman G., Hoffman T.J., Volkert W.A., Smith C.J. In Vitro and In Vivo Evaluation of Alexa Fluor 680-Bombesin[7-14]NH(2) Peptide Conjugate, a High-Affinity Fluorescent Probe with High Selectivity for the Gastrin-Releasing Peptide Receptor. Mol Imaging. 2007; **6** (3):171–80. PubMed PMID: 17532883.
2. Berlier J.E., Rothe A., Buller G., Bradford J., Gray D.R., Filanoski B.J., Telford W.G., Yue S., Liu J., Cheung C.Y., Chang W., Hirsch J.D., Beechem J.M., Haugland R.P., Haugland R.P. Quantitative comparison of long-wavelength Alexa Fluor dyes to Cy dyes: fluorescence of the dyes and their bioconjugates. J Histochem Cytochem. 2003; **51** (12):1699–712. PubMed PMID: 14623938.
3. Smith C.J., Gali H., Sieckman G.L., Higginbotham C., Volkert W.A., Hoffman T.J. Radiochemical investigations of (99m)Tc-N(3)S-X-BBN[7-14]NH(2): an in vitro/in vivo structure-activity relationship study where X = 0-, 3-, 5-, 8-, and 11-carbon tethering moieties. Bioconjug Chem. 2003; **14** (1):93–102. PubMed PMID: 12526698.
4. Mantey S., Frucht H., Coy D.H., Jensen R.T. Characterization of bombesin receptors using a novel, potent, radiolabeled antagonist that distinguishes bombesin receptor subtypes. Mol Pharmacol. 1993; **43** (5):762–74. PubMed PMID: 7684815.
5. Benya R.V., Kusui T., Pradhan T.K., Battey J.F., Jensen R.T. Expression and characterization of cloned human bombesin receptors. Mol Pharmacol. 1995; **47** (1):10–20. PubMed PMID: 7838118.
6. Reubi J.C., Wenger S., Schmuckli-Maurer J., Schaer J.C., Gugger M. Bombesin receptor subtypes in human cancers: detection with the universal radioligand (125)I-[D-TYR(6), beta-ALA(11), PHE(13), NLE(14)] bombesin(6-14). Clin Cancer Res. 2002; **8** (4):1139–46. PubMed PMID: 11948125.
7. Smith C.J., Volkert W.A., Hoffman T.J. Radiolabeled peptide conjugates for targeting of the bombesin receptor superfamily subtypes. Nucl Med Biol. 2005; **32** (7):733–40. PubMed PMID: 16243649.
8. Biddlecombe G.B., Rogers B.E., de Visser M., Parry J.J., de Jong M., Erion J.L., Lewis J.S. Molecular imaging of gastrin-releasing peptide receptor-positive tumors in mice using 64Cu- and 86Y-DOTA-(Pro1,Tyr4)-bombesin(1-14). Bioconjug Chem. 2007; **18** (3):724–30. PubMed PMID: 17378600.
9. Nock B., Nikolopoulou A., Chiotellis E., Loudos G., Maintas D., Reubi J.C., Maina T. [99mTc]Demobesin 1, a novel potent bombesin analogue for GRP receptor-targeted tumour imaging. Eur J Nucl Med Mol Imaging. 2003; **30** (2):247–58. PubMed PMID: 12552343.
10. Smith C.J., Volkert W.A., Hoffman T.J. Gastrin releasing peptide (GRP) receptor targeted radiopharmaceuticals: a concise update. Nucl Med Biol. 2003; **30** (8):861–8. PubMed PMID: 14698790.
11. Breeman W.A., Bakker W.H., De Jong M., Hofland L.J., Kwekkeboom D.J., Kooij P.P., Visser T.J., Krenning E.P. Studies on radiolabeled somatostatin analogues in rats and in patients. Q J Nucl Med. 1996; **40** (3):209–20. PubMed PMID: 8961800.
12. Zhang X., Cai W., Cao F., Schreibmann E., Wu Y., Wu J.C., Xing L., Chen X. 18F-labeled bombesin analogs for targeting GRP receptor-expressing prostate cancer. J Nucl Med. 2006; **47** (3):492–501. PubMed PMID: 16513619.

13. Massoud T.F., Gambhir S.S. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev. 2003; **17** (5):545–80. PubMed PMID: 12629038.
14. Bremer C., Ntziachristos V., Weissleder R. Optical-based molecular imaging: contrast agents and potential medical applications. Eur Radiol. 2003; **13** (2):231–43. PubMed PMID: 12598985.
15. Panchuk-Voloshina N., Haugland R.P., Bishop-Stewart J., Bhalgat M.K., Millard P.J., Mao F., Leung W.Y., Haugland R.P. Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. J Histochem Cytochem. 1999; **47** (9):1179–88. PubMed PMID: 10449539.
16. Smith C.J., Sieckman G.L., Owen N.K., Hayes D.L., Mazuru D.G., Volkert W.A., Hoffman T.J. Radiochemical investigations of [188Re(H2O)(CO)3-diaminopropionic acid-SSS-bombesin(7-14)NH2]: syntheses, radiolabeling and in vitro/in vivo GRP receptor targeting studies. Anticancer Res. 2003; **23** (1A):63–70. PubMed PMID: 12680195.
17. von Guggenberg, E., H. Dietrich, I. Skvortsova, M. Gabriel, I.J. Virgolini, and C. Decristoforo, (99m)Tc-labelled HYNIC-minigastrin with reduced kidney uptake for targeting of CCK-2 receptor-positive tumours. Eur J Nucl Med Mol Imaging, 2007.