

[0008] In view of the foregoing, there is a need in the art for methods of measuring or quantitating the levels of metabolites of the tryptophan, serotonin and kynurenine pathways in a biological sample from a subject. In addition, methods are need for diagnosing IBS in an individual by monitoring the brain-gut-microbiome axis. Assays are needed to assess whether various metabolic and catabolic pathways are functioning properly. The present invention satisfies these and other needs.

BRIEF SUMMARY OF THE INVENTION

[0009] In one aspect, provided herein is an isolated antibody or antibody fragment thereof that specifically binds to 5-hydroxyindole-3-acetic acid (5-HIAA) and has less than 1% cross-reactivity to one or more members selected from the group consisting of tryptophan (Trp), serotonin (5-HT), 5-hydroxytryptophan (5-HTP), kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid (QUIN), anthranilic acid (ANA), serotonin-O-sulfate, serotonin-O-phosphate, and melatonin (MT). The isolated antibody or purified antibody can be a polyclonal antibody or a monoclonal antibody. In some embodiments, the isolated antibody or purified antibody is a chimeric or a humanized antibody. The isolated or purified antibody fragment thereof can be a Fab fragment, a Fab' fragment or F(ab)'₂ fragment.

[0010] In some embodiments, the antibody or antibody fragment thereof against 5-HIAA is produced by the hybridoma cell line deposited on November 17, 2015 under ATCC Accession Number PTA-122671 and designated 1204-10G6F11H3.

[0011] In one embodiment, the antibody or antibody fragment thereof is produced by immunizing an animal with an immunogen comprising a 5-HIAA derivative conjugated to a carrier protein under conditions such that immune cells of the animal produce an antibody or antibody fragment thereof that specifically binds to 5-HIAA; and isolating the antibody or antibody fragment thereof from the animal. The animal can be a goat, rabbit or mouse. In some embodiments, the 5-HIAA derivative comprises a benzoxazole derivative of 5-HIAA.

[0012] In another aspect, provided herein is an isolated antibody or antibody fragment thereof that specifically binds to melatonin (MT) and has less than 1% cross-reactivity to one or more members selected from the group consisting of tryptophan (Trp), serotonin (5-HT), 5-hydroxytryptophan (5-HTP), 5-hydroxyindole-3-acetic acid (5-HIAA), kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid (QUIN), anthranilic acid (ANA), serotonin-O-sulfate, and serotonin-O-

phosphate. The isolated antibody or purified antibody can be a polyclonal antibody or a monoclonal antibody. In some embodiments, the isolated antibody or purified antibody is a chimeric or a humanized antibody. The isolated or purified antibody fragment thereof can be a Fab fragment, a Fab' fragment or F(ab)₂ fragment.

5 **[0013]** In some embodiments, the antibody or antibody fragment thereof against melatonin is produced by the hybridoma cell line deposited on November 17, 2015 under ATCC Accession Number PTA-122669 and designated 1212-6C1E2F7.

10 **[0014]** In one embodiment, the antibody or antibody fragment thereof is produced by immunizing an animal with an immunogen comprising melatonin conjugated to a carrier protein under conditions such that immune cells of the animal produce an antibody or antibody fragment thereof that specifically binds to melatonin; and isolating the antibody or antibody fragment thereof from the animal. The animal can be a goat, rabbit or mouse.

15 **[0015]** In yet another aspect, provided herein is an isolated antibody or antibody fragment thereof that specifically binds to kynurenic acid (KYNA), which has less than 1% cross-reactivity to one or more members selected from the group consisting of tryptophan (Trp), serotonin (5-HT), 5-hydroxytryptophan (5-HTP), 5-hydroxyindole-3-acetic acid (5-HIAA), kynurenine (KYN), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid (QUIN), anthranilic acid (ANA), serotonin-O-sulfate, serotonin-O-phosphate, and melatonin (MT). The isolated antibody or purified antibody can be a polyclonal antibody or a monoclonal antibody. In some embodiments, the isolated antibody or purified antibody is a chimeric or a humanized antibody. The isolated or purified antibody fragment thereof can be a Fab fragment, a Fab' fragment or F(ab)₂ fragment.

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25 **[0016]** In some embodiments, the antibody or antibody fragment thereof against kynurenic acid is produced by the hybridoma cell line deposited on November 17, 2015 under ATCC Accession Number PTA-122670 and designated 1194-6H5B11A7.

30 **[0017]** In one embodiment, the antibody or antibody fragment thereof is produced by immunizing an animal with an immunogen comprising kynurenic acid (KYNA) conjugated to a carrier protein under conditions such that immune cells of the animal produce an antibody or antibody fragment thereof that specifically binds to KYNA; and isolating the antibody or antibody fragment thereof from the animal. The animal can be a goat, rabbit or mouse.

[0018] In some embodiments, any one of the isolated antibodies or antibody fragments thereof described herein has a detectable label.

[0019] In some embodiments, any one of the isolated antibodies or antibody fragments thereof described herein is immobilized on a solid substrate.

5 **[0020]** In one aspect, provided herein is a hybridoma cell line that produces and secretes monoclonal antibodies which selectively bind to 5-HIAA and that has been deposited under ATCC Accession No. PTA-122671 on November 17, 2015 and designated 1204-10G6F11H3.

[0021] In some aspects, provided herein is a hybridoma cell line that produces and secretes monoclonal antibodies which selectively bind to melatonin and that has been deposited under
10 ATCC Accession No. PTA-122669 on November 17, 2015 and designated 1212-6C1E2F7.

[0022] In one aspect, provided herein is a hybridoma cell line that produces and secretes monoclonal antibodies which selectively bind to kynurenic acid and that has been deposited under ATCC Accession No. PTA-122670 on November 17, 2015 and designated 1194-6H5B11A7.

15 **[0023]** Also provided herein is a method for detecting the level of 5-HIAA in a sample from a patient suspected of having irritable bowel syndrome using an immunoassay. The method comprises: (a) contacting the isolated antibody or antibody fragment thereof described above, a sample obtained from the patient, and immobilized 5-HIAA under suitable conditions to form a complex comprising the isolated antibody or antibody fragment thereof and 5-HIAA present in
20 the sample or the immobilized 5-HIAA; (b) detecting the level of antibody or antibody fragment thereof bound to the complex comprising the immobilized 5-HIAA; and (c) calculating the level of 5-HIAA in the sample based on the level of antibody or antibody fragment thereof from step (b).

[0024] In some embodiments, the isolated antibody or antibody fragment thereof, the sample, and the immobilized 5-HIAA are contacted simultaneously. In other embodiments, the isolated antibody or antibody fragment thereof, the sample, and the immobilized 5-HIAA are contacted sequentially. The immunoassay can be an ELISA such as a competitive ELISA.

[0025] In one aspect, provided herein is a method for detecting the level of melatonin in a sample from a patient suspected of having irritable bowel syndrome using an immunoassay.
30 The method comprises: (a) contacting the isolated antibody or antibody fragment thereof described above, a sample obtained from the patient, and immobilized melatonin under

hydroxykynurenine 146, 3-hydroxyanthranilic acid 149, quinolinic acid 160, and xanthurenic acid 148 of FIG. 1.

5 **[0124]** In one aspect, the present invention provides isolated or purified antibodies to metabolite conjugates. Firstly, a metabolite or stable derivative thereof can be prepared. Next, a carrier protein such as BSA is conjugated to the derivative. Antibodies against the metabolite or stable derivative thereof were made by injecting the conjugate into a mammal such as a rabbit, mouse, sheep, chicken, goat and the like. Thereafter, the biotinylated haptan can be used to test the reactivity, binding activity, specificity, and/or sensitivity of the antibody so produced.

10 **[0125]** In other aspects, the present invention provides methods for making antibodies, *e.g.*, polyclonal antibodies or monoclonal antibodies that specifically bind to a serotonin metabolite. The method comprises: (a) providing an immunogen comprising a derivative selected from the group consisting of serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), and 5-hydroxy tryptophan (5-HTP) each derivative conjugated to a carrier protein; (b) immunizing an animal
15 with the immunogen under conditions such that the immune system of the animal makes the antibodies; and (c) removing the antibodies from the animal.

20 **[0126]** In one aspect, the antibody generated according to the method provided herein can be removed or isolated from serum or cell culture supernatant using the conjugates of the present invention. For example, in one aspect, a 5-HIAA compound, a conjugate thereof or a derivative thereof can be used to remove the antibody from the serum of an immunized animal, such as an immunized goat, rabbit or mouse. The antibody can be purified by selectively enriching or specifically isolating antibodies of interest from serum, ascites fluid, cell culture supernatant or media, and the like. For example, an affinity method such as an antigen-specific affinity method or an immunoglobulin class-specific affinity method can be used to isolate antibodies
25 of interest. The biotinylated 5-HIAA compound can be used to remove their corresponding antibodies from a mammal (such as a rabbit, mouse or goat).

30 **[0127]** In some aspect, the present invention provides an isolated or purified monoclonal antibody that is immunoreactive to 5-HIAA and is produced by a hybridoma cell line deposited at American Type Culture Collection (ATCC[®]) on November 17, 2015 under ATCC Accession No. PTA-122671 and designated 1204-10G6F11H3. Such an antibody has substantially no cross-reactivity to other structurally similar metabolites or compounds of the tryptophan, serotonin, and kynurenine pathway including tryptophan 122, 5-

than 1% cross-reactivity, *e.g.*, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1% or 0% cross-reactivity to structurally similar compounds in the tryptophan, serotonin and kynurenine pathways. In some instances, the anti-melatonin antibody or fragment thereof has substantially no cross-reactivity to metabolites or compounds of the tryptophan, serotonin and kynurenine pathways that are structurally similar to melatonin including tryptophan 122, 5-hydroxytryptophan 125, serotonin 101, 5-hydroxyindole acetic acid 115, kynurenine 131, kynurenic acid 135, anthranilic acid 140, 3-hydroxykynurenine 146, 3-hydroxyanthranilic acid 149, quinolinic acid 160, and xanthurenic acid 148 of FIG. 1. Provided herein are polyclonal antibodies and monoclonal antibodies that are specifically immunoreactive to melatonin.

- 10 **[0150]** In one aspect, the antibody or antigen binding fragment thereof generated from a mammal can be removed or separated from the serum using the conjugates of the present invention. In some cases, biotinylated melatonin or melatonin conjugated to another biomolecule or compound can be used to remove anti-melatonin antibodies from a mammal. Detailed descriptions of purification methods are disclosed below.
- 15 **[0151]** In some aspect, the present invention provides an isolated or purified monoclonal antibody that is immunoreactive to melatonin and is produced by a hybridoma cell line deposited at American Type Culture Collection (ATCC[®]) on November 17, 2015 under ATCC Accession No. PTA-122669 and designated 1212-6C1E2F7.

C. Kynurenine Pathway Metabolite – Kynurenic Acid Haptens

- 20 **[0152]** Kynurenine pathway metabolites play a role in the mechanism of visceral pain and have been linked to low level immune activation in IBS. Only 1% of dietary tryptophan is converted to serotonin and more than 95% is metabolized to kynurenines. Both kynurenine levels and the “kynurenine:tryptophan ratio” are significantly increased in patients with IBS. Typically, IBS patients show a decreased concentration of kynurenic acid (KYNA) and an increase in anthranilic acid (ANA) and 3-hydroxyanthranilic acid. Tryptophan metabolism along the kynurenine pathway is inhibited in patient with IBS-D. The present invention provides immunoassays to determine the levels of tryptophan and kynurenine pathway metabolites, which is of diagnostic importance for determining the status of IBS patients.

- 30 **[0153]** Provided herein are stable kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid and anthranilic acid haptens that can be

hapten can also be conjugated to other biomolecules. For instance, to test the affinity and specificity of the antibody thus produced, a hapten can be linked conjugated or linked to biotin to make a biotinylated hapten *e.g.*, a biotinylated KYNA.

[0169] In another aspect, the present invention provides an isolated or purified antibody or antigen binding fragment thereof that specifically binds to kynurenic acid, and wherein the antibody has less than 1% cross-reactivity to one or more members selected from the group consisting of tryptophan 122, 5-hydroxytryptophan 125, serotonin 101, 5-hydroxyindole acetic acid (5-HIAA) 115, kynurenine 131, anthranilic acid 140, 3-hydroxykynurenine 146, 3-hydroxyanthranilic acid 149, quinolinic acid 160, xanthurenic acid 148 and melatonin 120 of

FIG. 1

[0170] In some aspect, the present invention provides an isolated or purified monoclonal antibody that is immunoreactive to KYNA and is produced by a hybridoma cell line deposited at American Type Culture Collection (ATCC®) on November 17, 2015 under ATCC Accession No. PTA-122670 and designated 1194-6H5B11A7. Such an antibody has substantially no cross-reactivity to other structurally similar metabolites or compounds of the tryptophan, serotonin, and kynurenine pathway.

D. Detecting Metabolites in a Biological Sample Using an Immunoassay

[0171] In some aspects, the present disclosure provides assay methods and kits for detecting, measuring or quantitating the level of melatonin in a biological sample from a subject, such as a human subject. In some embodiments, the human subject has a condition associated with a higher or lower level of melatonin, 5-HIAA, and/or kynurenic acid compared to a normal subject. In some instances, the condition is irritable bowel syndrome including any one of the subtypes: IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS (IBS-M) and unsubtyped IBS (IBS-U). The method can include using an antibody against 5-HIAA, biotinylated 5-HIAA, an antibody against kynurenic acid, biotinylated kynurenic acid, an antibody against melatonin, biotinylated melatonin, and any combination thereof.

[0172] In some aspects, the present invention provides a method for assaying, measuring or detecting the presence or level of a serotonin metabolite in a biological sample such as a fluid or tissue sample from a mammal, *e.g.*, a human. In some embodiments, the serotonin metabolite is 5-hydroxyindole acetic acid (5-HIAA). In some instances, the method includes measuring or quantitating the amount or concentration of 5-HIAA in a biological sample

fluorescence polarization immunoassay, an immunoassay comprising FRET, enzyme linked immunosorbent assay (ELISA) or CEER™.

[0183] Any ELISA known in the art as useful for hapten detection can be utilized for the instant assays. ELISA for haptens generally utilize a competitive format, *i.e.*, where the hapten
5 (a metabolite) in the sample competes with a labeled hapten (*e.g.*, a biotin-hapten or enzyme-hapten conjugate) for anti-hapten antibody binding sites such that less labeled hapten is bound when there is more hapten in the sample. Thus, in these competitive assays, an increasing amount of hapten in the sample results in less enzyme bound to the solid phase, and consequently less detectable signal. In such competitive assays the sample can be added with
10 the labeled hapten to compete directly for antibody binding sites, or the sample and labeled hapten can be added sequentially such that the labeled hapten simply binds where the sample hapten is not bound. In some embodiments, the ELISA is a direct competitive ELISA, or an indirect competitive ELISA.

[0184] In one embodiment, the antibodies produced herein are bound to a solid phase, either
15 directly or indirectly, the latter being where the solid phase is coated with an anti-antibody (for example goat antibodies that bind to rabbit IgG antibodies (goat anti-rabbit IgG) and the antibodies are bound to the anti-antibody. The anti-antibodies are also known as secondary antibodies. In these assays, the sample and a labeled hapten are added to the solid phase to compete with antibody binding sites on the coated solid phase. After washing, the signal is
20 generated, which measures the amount of labeled hapten that is bound to the solid phase.

[0185] Provided herein are kits for performing the assay methods described above. In some embodiments, the kit comprises an antibody that specifically binds to 5-HIAA, *e.g.*, an anti-5HIAA monoclonal antibody or polyclonal antibody, and optionally a biotinylated 5-HIAA derivative. The monoclonal antibody against 5-HIAA may be produced by the hybridoma
25 clone having the ATCC Accession No. PTA-122671, deposited on November 17, 2015, and designated 1204-10G6F11H3.

[0186] In other embodiments, the kit comprises an antibody that specifically binds to melatonin, *e.g.*, an anti-melatonin monoclonal antibody or polyclonal antibody, and optionally biotinylated melatonin. The monoclonal antibody may be produced by the hybridoma clone
30 having the ATCC Accession No. PTA-122669, deposited on November 17, 2015, and designated 1212-6C1E2F7.

[0187] In yet other embodiments, the kit comprises an antibody that specifically binds to kynurenic acid, *e.g.*, an anti-kynurenic acid monoclonal antibody or polyclonal antibody, and optionally biotinylated kynurenic acid. The monoclonal antibody may be produced by the hybridoma clone having the ATCC Accession No. PTA-122670, deposited on November 17, 2015, and designated 1194-6H5B11A7.

[0188] In some instances, the kit also includes an instruction manual for performing the assay methods discussed herein. The kit may include a standard control metabolite such as a 5-HIAA standard control, melatonin standard control or a kynurenic acid standard control. In some embodiments, the standard control metabolite comprises a preselected or known concentration of the metabolite of interest.

E. Polyclonal Antibodies

[0189] Polyclonal antibodies provided herein can be of any isotype such as one of the major antibody isotypes: IgA, IgD, IgE, IgG, and IgM. In some embodiments, the antibody can be classified as an IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ or IgA₂ antibody. In some instances, the antibody has a kappa (κ) light chain or a lambda (λ) light chain.

[0190] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of an antigen of the present invention and an adjuvant. It may be useful to conjugate the antigen of interest to a carrier protein that is immunogenic in the species to be immunized using a bifunctional or derivatizing agent. Non-limiting examples of bifunctional or derivatizing agents include maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (conjugation through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, and R¹N=C=NR, wherein R and R¹ are different alkyl groups.

[0191] Animals are immunized against the antigens of the present invention or an immunogenic conjugate or derivative thereof by combining, *e.g.*, 100 μ g (for rabbits) or 5 μ g (for mice) of the antigen or conjugate with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with about 1/5 to 1/10 the original amount of conjugate in Freund's incomplete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the animals are bled and the serum is assayed for antibody titer. Animals are typically boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugation to a different immunogenic antigen and/or through a different cross-

linking reagent may be used. In certain instances, aggregating agents such as alum can be used to enhance the immune response. Detailed descriptions of methods for producing polyclonal antibodies is found in, *e.g.*, *Antibodies, A Laboratory Manual*, Harlow and Lane, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988).

5 **F. Monoclonal Antibodies**

10 **[0192]** Monoclonal antibodies provided herein can be of any isotype such as one of the major antibody isotypes: IgA, IgD, IgE, IgG, and IgM. In some embodiments, the antibody can be classified as an IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ or IgA₂ antibody. In some instances, the antibody has a kappa (κ) light chain or a lambda (λ) light chain. In some embodiments, the monoclonal antibody against 5-HIAA or the monoclonal antibody produced by hybridoma clone having ATCC Accession No. PTA-122671, deposited on November 17, 2015 and designated 1204-10G6F11H3 is an IgG1κ antibody. In other embodiments, the monoclonal antibody against melatonin or the monoclonal antibody produced by hybridoma clone having ATCC Accession No. PTA-122669, deposited on November 17, 2015 and designated 1212-6C1E2F7 is an IgG₃κ antibody. In yet other embodiments, the monoclonal antibody against KYNA or the monoclonal antibody produced by hybridoma clone having ATCC Accession No. PTA-122670, deposited on November 12, 2015 and designated 1194-6H5B11A7 is an IgG1κ antibody.

20 **[0193]** Monoclonal antibodies are generally obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies. For example, monoclonal antibodies can be made using the hybridoma method described by Kohler *et al.*, *Nature*, 256:495 (1975) or by any recombinant DNA method known in the art (*see, e.g.*, U.S. Patent No. 4,816,567).

25 **[0194]** In the hybridoma method, a mouse or other appropriate host animal (*e.g.*, hamster) is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies which specifically bind to the polypeptide of interest used for immunization. Alternatively, lymphocytes are immunized *in vitro*. The immunized lymphocytes are then fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form hybridoma cells (*see, e.g.*, Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, pp. 59-103 (1986)). The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or

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WHAT IS CLAIMED IS:

- 1 **1.** An isolated antibody or antibody fragment thereof that specifically
2 binds to 5-hydroxyindole-3-acetic acid (5-HIAA) and has less than 1% cross-reactivity to one
3 or more members selected from the group consisting of tryptophan (Trp), serotonin (5-HT), 5-
4 hydroxytryptophan (5-HTP), kynurenine (KYN), kynurenic acid (KYNA), 3-
5 hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid (QUIN),
6 anthranilic acid (ANA), serotonin-O-sulfate, serotonin-O-phosphate, and melatonin (MT).
- 1 **2.** The isolated antibody or antibody fragment thereof of claim **1**, wherein
2 the antibody is a polyclonal antibody or a monoclonal antibody.
- 1 **3.** The isolated antibody or antibody fragment thereof of claim **1**, wherein
2 the antibody is a chimeric or a humanized antibody.
- 1 **4.** The isolated antibody or antibody fragment thereof of claim **1**, wherein
2 the antibody fragment is a Fab fragment, a Fab' fragment or F(ab)₂ fragment.
- 1 **5.** The isolated antibody or antibody fragment thereof of claim **1**, wherein
2 the antibody or antibody fragment thereof is produced by the hybridoma cell line deposited
3 under ATCC Accession No. PTA-122671 on November 17, 2015 and designated 1204-
4 10G6F11H3.
- 1 **6.** The isolated antibody or antibody fragment thereof of claim **1**, wherein
2 the antibody or antibody fragment thereof is produced by immunizing an animal with an
3 immunogen comprising a 5-HIAA derivative conjugated to a carrier protein under conditions
4 such that immune cells of the animal produce an antibody or antibody fragment thereof that
5 specifically binds to 5-HIAA; and isolating the antibody or antibody fragment thereof from the
6 animal.
- 1 **7.** The isolated antibody or antibody fragment thereof of claim **6**, wherein
2 the animal is a goat, rabbit or mouse.
- 1 **8.** The isolated antibody or antibody fragment thereof of claim **6**, wherein
2 the 5-HIAA derivative comprises a benzoxazole derivative of 5-HIAA.
- 1 **9.** An isolated antibody or antibody fragment thereof that specifically
2 binds to melatonin (MT) and has less than 1% cross-reactivity to one or more members

3 selected from the group consisting of tryptophan (Trp), serotonin (5-HT), 5-hydroxytryptophan
4 (5-HTP), 5-hydroxyindole-3-acetic acid (5-HIAA), kynurenine (KYN), kynurenic acid
5 (KYNA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid
6 (QUIN), anthranilic acid (ANA), serotonin-O-sulfate, and serotonin-O-phosphate.

1 **10.** The isolated antibody or antibody fragment thereof of claim **9**, wherein
2 the antibody is a polyclonal antibody or a monoclonal antibody.

1 **11.** The isolated antibody or antibody fragment thereof of claim **9**, wherein
2 the antibody is a chimeric or a humanized antibody.

1 **12.** The isolated antibody or antibody fragment thereof of claim **9**, wherein
2 the antibody fragment is a Fab fragment, a Fab' fragment or F(ab)₂ fragment.

1 **13.** The isolated antibody or antibody fragment thereof of claim **9**, wherein
2 the antibody or antibody fragment thereof is produced by the hybridoma cell line deposited
3 under ATCC Accession No. PTA-122669 on November 17, 2015 and designated 1212-
4 6C1E2F7.

1 **14.** The isolated antibody or antibody fragment thereof of claim **9**, wherein
2 the antibody or antibody fragment thereof is produced by immunizing an animal with an
3 immunogen comprising melatonin conjugated to a carrier protein under conditions such that
4 immune cells of the animal produce an antibody or antibody fragment thereof that specifically
5 binds to melatonin; and isolating the antibody or antibody fragment thereof from the animal.

1 **15.** An isolated antibody or antibody fragment thereof that specifically
2 binds to kynurenic acid (KYNA), which has less than 1% cross-reactivity to one or more
3 members selected from the group consisting of tryptophan (Trp), serotonin (5-HT), 5-
4 hydroxytryptophan (5-HTP), 5-hydroxyindole-3-acetic acid (5-HIAA), kynurenine (KYN), 3-
5 hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid (QUIN),
6 anthranilic acid (ANA), serotonin-O-sulfate, serotonin-O-phosphate, and melatonin.

1 **16.** The isolated antibody or antibody fragment thereof of claim **15**, wherein
2 the antibody is a polyclonal antibody or a monoclonal antibody.

1 **17.** The isolated antibody or antibody fragment thereof of claim **15**, wherein
2 the antibody is a chimeric or a humanized antibody.

1 **18.** The isolated antibody or antibody fragment thereof of claim **15**, wherein
2 the antibody fragment is a Fab fragment, a Fab' fragment or F(ab)'₂ fragment.

1 **19.** The isolated antibody or antibody fragment thereof of claim **15**, wherein
2 the antibody or antibody fragment thereof is produced by the hybridoma cell line deposited
3 under ATCC Accession No. PTA-122670 on November 17, 2015 and designated 1194-
4 6H5B11A7.

1 **20.** The isolated antibody or antibody fragment thereof of claim **15**, wherein
2 the antibody or antibody fragment thereof is produced by immunizing an animal with an
3 immunogen comprising kynurenic acid (KYNA) conjugated to a carrier protein under
4 conditions such that immune cells of the animal produce an antibody or antibody fragment
5 thereof that specifically binds to KYNA; and isolating the antibody or antibody fragment
6 thereof from the animal.

1 **21.** The isolated antibody or antibody fragment thereof of any one of claims
2 1 to 20, which has a detectable label.

1 **22.** The isolated antibody or antibody fragment thereof of any one of claims
2 1 to 21, which is immobilized on a solid substrate.

1 **23.** A hybridoma cell line which produces and secretes monoclonal
2 antibodies which selectively bind to 5-HIAA and has been deposited under ATCC Accession
3 No. PTA-122671 on November 17, 2015 and designated 1204-10G6F11H3.

1 **24.** A hybridoma cell line which produces and secretes monoclonal
2 antibodies which selectively bind to melatonin and has been deposited under ATCC Accession
3 No. PTA-122669 on November 17, 2015 and designated 1212-6C1E2F7.

1 **25.** A hybridoma cell line which produces and secretes monoclonal
2 antibodies which selectively bind to kynurenic acid and has been deposited under ATCC
3 Accession No. PTA-122670 on November 17, 2015 and designated 1194-6H5B11A7.