The Kynurenine pathway.
Malaria today afflicts ~500 million people throughout the tropical world.

~1 million, mainly children, die each year
Fig. 1  NMDAR model showing binding sites for agonists and antagonists. The extracellular portions of NR1 and NR2 subunits consist of two domains, the modulatory domain and the agonist binding domain. Glycine and D-serine are agonists for NR1 subunit.

Mehdi Ghasemi, Steven C. Schachter

The NMDA receptor complex as a therapeutic target in epilepsy: a review

Epilepsy & Behavior Volume 22, Issue 4 2011 617 - 640

http://dx.doi.org/10.1016/j.yebeh.2011.07.024
Malaria today afflicts ~500 million people throughout the tropical world.

~1 million, mainly children, die each year
KMO inhibition protects mice against cerebral malaria

- *Plasmodium berghei* infected mice die in 7-9 days showing neurological dysfunction
- Treat with 200mg/Kg Ro-61-8048 over 12 days (every 1 or 2 days); parasitaemia the same but no neurological dysfunction
- Euthanased at 21 days, pronounced anaemia
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Picolinic acid</th>
<th>Kynurenic acid</th>
<th>Anthranilic acid</th>
<th>Quinolinic acid</th>
<th>MIP-1α</th>
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</thead>
<tbody>
<tr>
<td>Control + vehicle</td>
<td>309 ± 49</td>
<td>1.3 ± 0.5</td>
<td>2.0 ± 0.3</td>
<td>144 ± 34</td>
<td>0.58 ± 0.06</td>
</tr>
<tr>
<td>Control + Ro-61-8048</td>
<td>265 ± 32</td>
<td>10.2 ± 1.5</td>
<td>76.9 ± 23.3</td>
<td>150 ± 32</td>
<td>0.57 ± 0.09</td>
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<tr>
<td><em>P. berghei</em> ANKA + vehicle</td>
<td>1,077 ± 265&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5 ± 0.9</td>
<td>2.7 ± 0.6</td>
<td>186 ± 28</td>
<td>4.11 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. berghei</em> ANKA + Ro-61-8048</td>
<td>300 ± 72</td>
<td>35.6 ± 6.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>487 ± 96&lt;sup&gt;d&lt;/sup&gt;</td>
<td>170 ± 29</td>
<td>2.26 ± 0.28&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 2. Molecular interplay between endothelium and astrocytes, with some functional consequences: the example of cerebral malaria (CM). In this case the pathogen (*Plasmodium falciparum*)-infected red blood cell...
Trypanosomes in blood
Trypanosoma brucei brucei GVR35 murine model

Well established & characterised
International standard for CNS disease investigations

T.b. GVR35

• Parasites proliferate in the haemo-lymphatic system and peripheral tissues
• Infection can be cured by treatment with stage 1 drugs

7 14 21 28 35

Acute Infection
Early CNS stage
Late CNS stage

Monitor parasitemia

Trypanosomes identified in the inter-ventricular foramen

• Parasites established within the CNS
• Stage 1 treatments are no longer effective
Evaluating chemotherapy

**T.b. GVR35**

**Administer Drugs**

- Monitor
- Up to 180 days
- +ve → Treatment unsuccessful
- -ve
- Up to 180 days
- +ve

**Parasites in CNS**

- -ve
- Takes too long

**Treatment successful**

**Treatment unsuccessful**
Develop improved models to assess drugs against Human African Trypanosomiasis

In Vivo Imaging System (IVIS)  Multi-Photon Laser Scanning Microscopy (MPLSM)  Magnetic Resonance Imaging (MRI)
Use of IVIS imaging to monitor CNS stage disease

<table>
<thead>
<tr>
<th>Day 36</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 22</th>
<th>Day 28</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Flux (p/sec)</td>
<td>4x10^5</td>
<td>2x10^8</td>
<td>5x10^8</td>
<td>6x10^8</td>
<td>1x10^9</td>
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<tr>
<td>Parasites/ ml of blood</td>
<td>2.7x10^8</td>
<td>3x10^6</td>
<td>3.5x10^6</td>
<td>3.7x10^7</td>
<td>2x10^7</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 22</th>
<th>Day 28</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Flux (p/sec)</td>
<td>2.7x10^7</td>
<td>4x10^7</td>
<td>2.3x10^8</td>
<td>4.4x10^8</td>
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<tr>
<td>Parasites/ ml of blood</td>
<td>2.7x10^7</td>
<td>4x10^7</td>
<td>2.3x10^8</td>
<td>4.4x10^8</td>
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GVR35 WT

GVR35 + LUC2
Use of IVIS imaging to monitor CNS stage disease

Organs of GVR35-infected mice imaged *ex vivo*

Day 35 post infection

<table>
<thead>
<tr>
<th>WT</th>
<th>LUC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td><img src="image1" alt="Brain WT" /> <img src="image2" alt="Brain LUC2" /></td>
</tr>
<tr>
<td>Spleen</td>
<td><img src="image3" alt="Spleen WT" /> <img src="image4" alt="Spleen LUC2" /></td>
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<tr>
<td>Liver</td>
<td><img src="image5" alt="Liver WT" /> <img src="image6" alt="Liver LUC2" /></td>
</tr>
<tr>
<td>Heart</td>
<td><img src="image7" alt="Heart WT" /> <img src="image8" alt="Heart LUC2" /></td>
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<tr>
<td>Lungs</td>
<td><img src="image9" alt="Lungs WT" /> <img src="image10" alt="Lungs LUC2" /></td>
</tr>
<tr>
<td>Kidney</td>
<td><img src="image11" alt="Kidney WT" /> <img src="image12" alt="Kidney LUC2" /></td>
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<tr>
<td>Eyes</td>
<td><img src="image13" alt="Eyes WT" /> <img src="image14" alt="Eyes LUC2" /></td>
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<table>
<thead>
<tr>
<th>WT</th>
<th>LUC2</th>
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</thead>
<tbody>
<tr>
<td>Axillary LN</td>
<td><img src="image15" alt="Axillary LN WT" /> <img src="image16" alt="Axillary LN LUC2" /></td>
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<tr>
<td>Inguinal LN</td>
<td><img src="image17" alt="Inguinal LN WT" /> <img src="image18" alt="Inguinal LN LUC2" /></td>
</tr>
<tr>
<td>Brachial LN</td>
<td><img src="image19" alt="Brachial LN WT" /> <img src="image20" alt="Brachial LN LUC2" /></td>
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<tr>
<td>Cervical LN</td>
<td><img src="image21" alt="Cervical LN WT" /> <img src="image22" alt="Cervical LN LUC2" /></td>
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<tr>
<td>Mesenteric LN</td>
<td><img src="image23" alt="Mesenteric LN WT" /> <img src="image24" alt="Mesenteric LN LUC2" /></td>
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<tr>
<td>Popliteal LN</td>
<td><img src="image25" alt="Popliteal LN WT" /> <img src="image26" alt="Popliteal LN LUC2" /></td>
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</tbody>
</table>
Brains of GVR35-infected mice imaged *ex vivo*

<table>
<thead>
<tr>
<th>Total Flux (p/sec)</th>
<th>GVR35 WT</th>
<th>GVR35 + LUC2</th>
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</thead>
<tbody>
<tr>
<td>4.3x10³</td>
<td><img src="image1" alt="Day 22" /></td>
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<tr>
<td>1.8x10⁴</td>
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<tr>
<td>5.4x10⁵</td>
<td><img src="image5" alt="Day 21" /></td>
<td><img src="image6" alt="Day 21" /></td>
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<tr>
<td>2.3x10⁶</td>
<td><img src="image7" alt="Day 28" /></td>
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<tr>
<td>3.6x10⁶</td>
<td><img src="image9" alt="Day 36" /></td>
<td><img src="image10" alt="Day 36" /></td>
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</tbody>
</table>

Day 13

olfactory bulbs  brainstem

Use of IVIS imaging to monitor CNS stage disease
Current Drugs

- Suramin
- Pentamidine
- Melarsoprol
- Eflornithine
- Nifurtimox
Melarsoprol treatment at day 21 post infection

<table>
<thead>
<tr>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 36</th>
<th>Day 57</th>
<th>Day 99</th>
<th>Day 127</th>
<th>Day 170</th>
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<tbody>
<tr>
<td>Total flux (p/sec)</td>
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<td>4.2x10^9</td>
<td>4.7x10^5</td>
<td>6.7x10^5</td>
<td>6.1x10^5</td>
<td>7.5x10^5</td>
</tr>
<tr>
<td>Parasites/ml of blood</td>
<td>3.3x10^6</td>
<td>7.9x10^6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Topical application: 3.6 mg x 3 days
### Diminazene aceturate (Berenil) treatment at day 21 post infection

**Total flux (p/sec)**

<table>
<thead>
<tr>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 41</th>
<th>Day 49</th>
<th>Day 56</th>
<th>Day 62</th>
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</thead>
<tbody>
<tr>
<td>8.4 x 10^8</td>
<td>9.2 x 10^7</td>
<td>5.1 x 10^5</td>
<td>5.2 x 10^5</td>
<td>6 x 10^5</td>
<td>5.8 x 10^5</td>
<td>5.1 x 10^5</td>
</tr>
</tbody>
</table>

**Parasites/ml of blood**

<table>
<thead>
<tr>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 41</th>
<th>Day 49</th>
<th>Day 56</th>
<th>Day 62</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6 x 10^6</td>
<td>5.6 x 10^6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

40mg/kg x 1 injected intraperitoneally
Imaging from the superficial meninges and into the brain parenchyma

Microscope objective

Skull bone (autofluorescent)

Blood labelled with dextran-rhodamine, 70 kD

Multi-photon Laser Scanning Microscopy

Level of tight junctions

Collagen
Trypanosomes in pial vessels in the brain *in vivo*

D3 post infection

*T. bb* 427 + mCherry

Blood vessels labelled with FITC-dextran
Trypanosomes in the superficial meninges \textit{in vivo}

The number of extravascular trypanosomes per unit area of superficial meninges depends on time since infection, not blood parasitaemia.
JM6 → Ro 61-8048 → KMO → ↑KYN → KYN → KYNA → EXCITOXICITY → Neuroprotection
The major cellular and molecular targets of kynurenic acid. Blockade of *N*-methyl-*D*-aspartic acid receptors was the first specific site of action to be identified. Roles in inflammation & cancer.
Figure 3   Potential sites at which kynurenine, long regarded as biologically inactive, may act to regulate the balance of cells produced in the immune system.

Trevor W. Stone, Nicholas Stoy, L. Gail Darlington

An expanding range of targets for kynurenine metabolites of tryptophan

Trends in Pharmacological Sciences null 2012 null

http://dx.doi.org/10.1016/j.tips.2012.09.006
The first enzyme in the kynurenine pathway, indolamine-2,3-dioxygenase (IDO), plays a key role in regulation of the immune system by virtue of its activation by mediators such as interferon-? . In addition to the effects of kynurenic acid...

Trevor W. Stone, Nicholas Stoy, L. Gail Darlington
Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection
Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection

structures are shown of several of the glutamate receptor blocking compounds based on the structure of kynurenic acid. Most act at the glycine-B receptor site on the NMDA receptor, the preferred site of action of kynurenic acid.
Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection

The structures are shown of the two main inhibitors of the kynurenine pathway that are neuroprotective and prevent excitotoxicity by blocking kynureninase or KMO.
<table>
<thead>
<tr>
<th>FORMULA</th>
<th>NAME</th>
<th>NAME</th>
<th>MAP</th>
<th>PATHWAY</th>
<th>GROUP</th>
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<th>D3_sup</th>
<th>D4_cell</th>
<th>D4_sup</th>
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<th>D6_cell</th>
<th>D6_sup</th>
<th>D7_cell</th>
<th>D7_sup</th>
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<td>C10H14N5O5</td>
<td>Adenyl sulfate</td>
<td>6</td>
<td>1</td>
<td>Nucleotide metabolism</td>
<td>Purine metabolism</td>
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<td>1.02</td>
<td>0.93</td>
<td>0.86</td>
<td>0.80</td>
<td>0.91</td>
<td>1.02</td>
<td>1.10</td>
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<td>C11H23NO3</td>
<td>[FA amino(11:0)] 11-amino-undecanoic acid</td>
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<td>Lipids: Fatty Acyls</td>
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<td>0.94</td>
<td>0.60</td>
<td>1.01</td>
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</tbody>
</table>

![L-Formylkynurenine](image-url)
Polyamine pathway

L-Arginine → Ornithine decarboxylase → Putrescine → Aminopropyltransferase → Spermidine

L-Ornithine → Ornithine decarboxylase → Putrescine

N-Acetylornithine → N-Acetylputrescine

Not Arginase
## Most significant effects (top 10 & bottom 10)

<table>
<thead>
<tr>
<th>Mass</th>
<th>RT</th>
<th>Formula</th>
<th>Isomers</th>
<th>Metabolite</th>
<th>Pathway</th>
<th>Cofactors 1</th>
<th>Cofactors 2</th>
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<td>C8H9NO</td>
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<td>2-Phenylacetamide</td>
<td>Phenylalanine metabolism</td>
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<td>161.047</td>
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<tr>
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<td>3-Indoleglycolaldehyde</td>
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<td>335.148</td>
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<td>Peptide(tri-)</td>
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<td>1.71</td>
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<tr>
<td>145.052</td>
<td>5.54</td>
<td>C9H7NO</td>
<td>7</td>
<td>3-Methylenedioxyindole</td>
<td>N/A</td>
<td>3.73</td>
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<tr>
<td>250.062</td>
<td>9.31</td>
<td>C8H14N2O5S</td>
<td>2</td>
<td>Glu-Cys</td>
<td>Peptide(di-)</td>
<td>3.45</td>
<td>1.38</td>
</tr>
<tr>
<td>236.079</td>
<td>10.5</td>
<td>C11H12N2O4</td>
<td>2</td>
<td>L-Formylkynurenine</td>
<td>Tryptophan metabolism</td>
<td>3.41</td>
<td>1.51</td>
</tr>
<tr>
<td>301.142</td>
<td>9.41</td>
<td>C16H19N3O3</td>
<td>3</td>
<td>Trp-Pro</td>
<td>Peptide(di-)</td>
<td>0.33</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Cofactors 1 is essential (NAD+)
Most significant effects (top 10 & bottom 10)

<table>
<thead>
<tr>
<th>Mass</th>
<th>RT</th>
<th>Formula</th>
<th>Isomers</th>
<th>Metabolite</th>
<th>Pathway</th>
<th>Cofactors 1</th>
<th>Cofactors 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>135.068</td>
<td>5.51</td>
<td>C8H9NO</td>
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<td>2-Phenylacetamide</td>
<td>Phenylalanine metabolism</td>
<td>5.71</td>
<td>1.22</td>
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<td>161.047</td>
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<td>C9H7NO2</td>
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<td>175.063</td>
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<td>C10H9NO2</td>
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<td>3-Indoleglycolaldehyde</td>
<td>Tryptophan metabolism</td>
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<tr>
<td>335.148</td>
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<td>C16H21N3O5</td>
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<td>Gly-Pro-Tyr</td>
<td>Peptide(tri-)</td>
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<td>1.71</td>
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<tr>
<td>189.042</td>
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<td>C10H7NO3</td>
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<td>Kynurenate</td>
<td>Tryptophan metabolism</td>
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<td>0.99</td>
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<tr>
<td>175.063</td>
<td>5.71</td>
<td>C10H9NO2</td>
<td>12</td>
<td>Indole-3-acetate</td>
<td>Tryptophan metabolism</td>
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<td>1.36</td>
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<td>196.063</td>
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<td>C12H8N2O</td>
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<td>2-hydroxyphenazine</td>
<td>Secondary Metabolism</td>
<td>4.24</td>
<td>1.62</td>
</tr>
<tr>
<td>145.052</td>
<td>5.54</td>
<td>C9H7NO</td>
<td>7</td>
<td>3-Methyleneoxindole</td>
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<td>3.73</td>
<td>1.3</td>
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<td>0.74</td>
</tr>
</tbody>
</table>

Multiple substrates
- Tryptophan
- Trp peptides

Multiple products
- Tryptophan pathway
- Oxidoreductase (or aminotransferase)

Cofactors 1 is essential (NAD+)
Tryptophan metabolism:
L-Tryptophan: possible enzyme

L-Tryptophan

Tryptophan 2,3-dioxygenase

O₂

L-Formylkynurenine

BYC1

BYC2

BYE1

BYE2
Tryptophan metabolism:
L-Tryptophan: possible enzyme

L-Tryptophan  \(\rightarrow\) Tryptophan 2'-dioxygenase  \(\rightarrow\) 3-Indoleglycolaldehyde

\[
\begin{align*}
\text{L-Tryptophan} & \quad \text{3-Indoleglycolaldehyde} \\
\text{BYC1} & \quad \text{BYC2} \\
\text{BYE1} & \quad \text{BYE2}
\end{align*}
\]
Tryptophan metabolism:
L-Tryptophan: possible enzyme

L-Tryptophan

\[
\text{C00078}
\]

Tryptophan dehydrogenase

\[
\text{NAD}^+ \quad \text{H}_2\text{O} \quad \text{NH}_3 \quad \text{H}^+ \quad \text{NADH}
\]

Indolepyruvate

\[
\text{C00331}
\]

? Not detected
Tryptophan metabolism:
Xanthurenone: possible enzyme

3-Hydroxy-L-kynurenine

Kynurenine aminotransferase

2-Oxoglutarate

L-Glutamate

Xanthurenone
Kynurenate: possible enzyme

L-Kynurenine

Kynurenine aminotransferase

Not detected
Altered kynurenine metabolism correlates with infarct volume in stroke

L. G. Darlington, G. M. Mackay, C. M. Forrest, N. Stoy, C. George and T. W. Stone

1Epsom General Hospital, Epsom, Surrey KT18 7EG, UK
2Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

*50 patients
*35 controls
*Bloods taken ASAP after presentation then at day 1, 2, 3, 4, 7 & 14
*HPLC, fluorescence to measure: tryp, kynurenine KA, AA and 3HAA
Altered kynurenine metabolism correlates with infarct volume in stroke

European Journal of Neuroscience
Altered kynurenine metabolism correlates with infarct volume in stroke

L. G. Darlington,1 G. M. Mackay,2 C. M. Forrest,2 N. Stoy,1 C. George1 and T. W. Stone2
1Epsom General Hospital, Epsom, Surrey KT18 7EG, UK
2Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Kynurenine pathway appears to elevate in these stroke patients, as measured in blood
Stroke Biomarkers

- 40 healthy controls
- 40 stroke
- 20 TIA
- Urine samples
Urinary Proteomics to Support Diagnosis of Stroke

Jesse Dawson¹*, Matthew Walters¹, Christian Delles¹, Harald Mischak¹,², William Mullen¹

¹Institute of Cardiovascular and Medical Sciences, College of Medicine, Veterinary & Life Sciences, University of Glasgow, Glasgow, United Kingdom, ²MosaicDiagnostics, Hannover, Germany

Metabolite profiling distinguishes stroke and TIA

Jesse Dawson
Colette Keenan
Karl Burgess
KNN Multi-marker Classifier

1. All metabolites (15) with $p<0.05$
2. All metabolites (6) with $p<0.01$
3. Common metabolites (6) with $p<0.05$
4. Common metabolites (4) with $p<0.01$

<table>
<thead>
<tr>
<th>Classifier</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Area Under ROC Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>All metabolites with $p&lt;0.05$</td>
<td>81.3%</td>
<td>69.0%</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>All metabolites with $p&lt;0.01$</strong></td>
<td><strong>81.3%</strong></td>
<td><strong>78.6%</strong></td>
<td><strong>0.80</strong></td>
</tr>
<tr>
<td>Common metabolites with $p&lt;0.05$</td>
<td>70.3%</td>
<td>78.6%</td>
<td>0.74</td>
</tr>
<tr>
<td>Common metabolites with $p&lt;0.01$</td>
<td>73.4%</td>
<td>78.6%</td>
<td>0.76</td>
</tr>
</tbody>
</table>

84.1% sensitivity and 85.7% specificity
\( \text{C}_{10}\text{H}_7\text{NO}_3 \quad \text{Kynuremate} \)
\( \text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_4 \quad \text{Formyl-N-acetyl-5-methoxykynurenamine} \)
\( \text{C}_9\text{H}_9\text{NO}_3 \quad \text{N-acetyl-anthrinilate} \)
\( \text{C}_9\text{H}_7\text{NO}_2 \quad \text{Dihydroxyquinoline} \)
\( \text{C}_6\text{H}_6\text{O}_2 \quad \text{Benzenediol} \)
\( \text{C}_6\text{H}_{10}\text{O}_5 \quad \text{L-Formyl-kynurenine} \)
The Kynurenine pathway

- Tryptophan → Serotonin
- N-formyl kynurenine → Formyl – N-acetyl-5-methoxy kynurenamine
- Kynurenine → 3-OH kynurenine → Benzenediol
- 3-OH anthranilic acid → N-acetyl anthranilic acid
- Anthranilic acid → 2-amino-3-carboxymuconate semialdehyde
- Anthranilic acid → Picolinic acid
- Kynurenic acid → Dihydroxyquinoline

Changed in stroke
Changed in TIA
GVR35 kynurenine
Jean Rodgers
Peter Kennedy
Trevor Stone

Stroke
Jesse Dawson
Colette Keenan
Karl Burgess

In vivo Imaging
Elmarie Myburgh
Ryan Ritchie
Jeremy Mottram

Enzyme work
Darren Creek
Felicity Lumb
Brunda Nijagal
Katharina Johnston