# 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives as potent inhibitors of adenovirus infection

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Spanish Adenovirus Network: from basic biology to nanobiomedicine

"Una manera de hacer Europa"

# HAdV infections: Clinical Relevance

- **HAdV** is responsible for diseases ranging from **acute respiratory and ocular infections** to more severe enteric diseases, but is **rarely associated with severe clinical symptoms** in otherwise healthy individuals

- **HAdV** is one of the more common causes of **potentially life-threatening viral diseases associated with transplantation** and a leading cause of increased infections in pediatric units

| Allo-HSCT | Rate of infection | Mortality rates |
|---|---|---|
| Pediatrics | 6%-42% | 60%-80% |
| Adults | 3%-15% | |

- With advances in **modern molecular techniques HAdV** has been increasingly found to be involved in **sporadic cases** and **outbreaks of severe CAP in healthy adults**

Despite its significant **clinical impact,** there are **no currently approved antiviral therapies to treat HAdV** infections

# High through-put screening (HTS) of a library of piperazine derivatives to identify compounds that restrict HAdV infection

In collaboration with the Department of Organic and Pharmaceutical Chemistry at **University of Seville**

Journal of Medicinal Chemistry

Article

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## New 4-Acyl-1-phenylaminocarbonyl-2-phenylpiperazine Derivatives as Potential Inhibitors of Adenovirus Infection. Synthesis, Biological Evaluation, and Structure–activity Relationships

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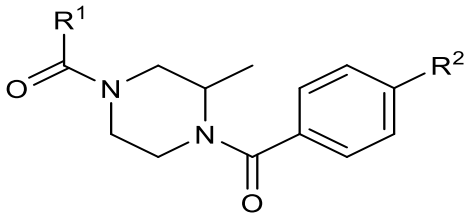
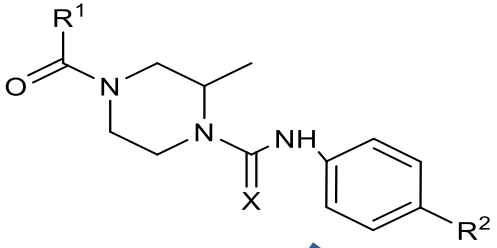
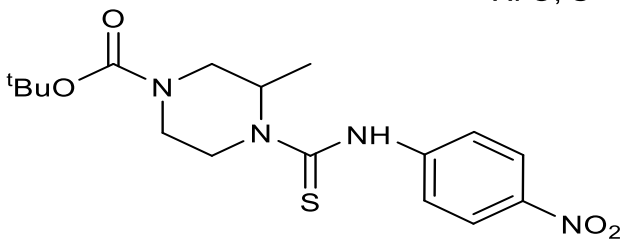
# Methyl-piperazine derivatives
# General chemical synthesis




$R^1$: O$^t$Bu, $^t$Bu, Ph, Benzofuran-2-yl
$R^2$: $NO_2$, $OCH_3$
X: O, S

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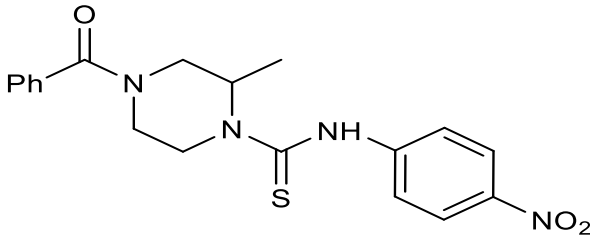



491

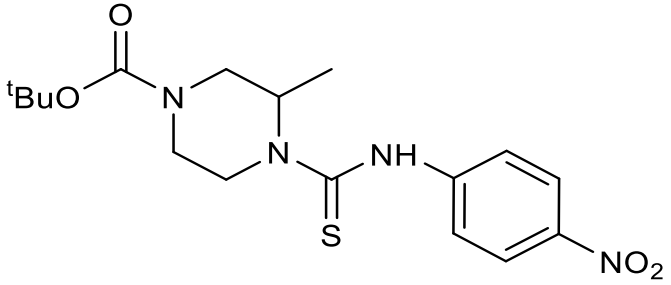
| Compound | Plaque assay inhibition (%) (10 µM) | $CC_{50}$ (µM) |
|---|---|---|
| **499** | 100 | 26.33 ± 1.59 |
| **491** | 92 | 31.44 ± 2.82 |

# Objectives

1. **Design, synthesis and antiviral evaluation of a new family of 4-acyl-1-phenylaminothiocarbonyl-piperazine derivatives**

2. **Characterization of the mechanisms of action of those derivatives showing significant anti-HAdV activity**

# Methyl-piperazine derivatives
# General chemical synthesis

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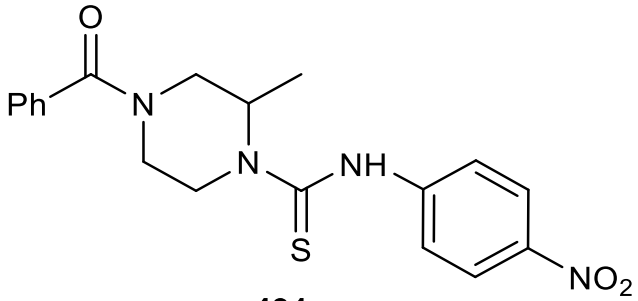


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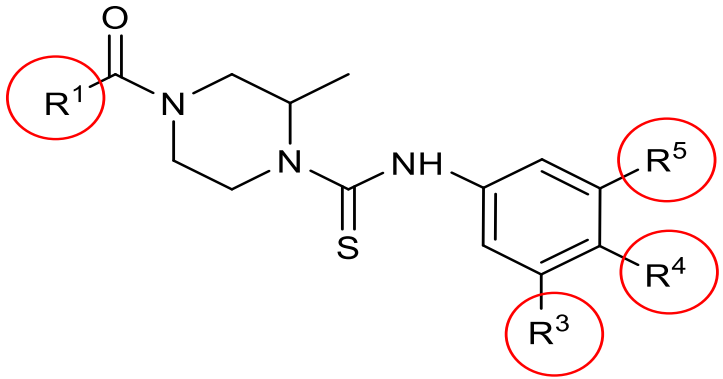
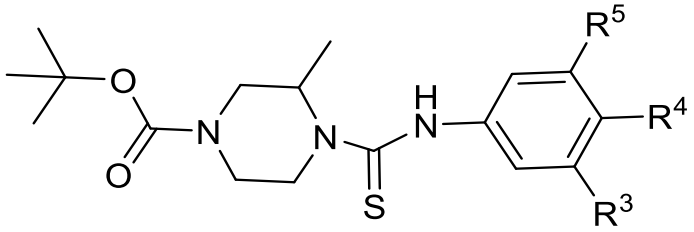


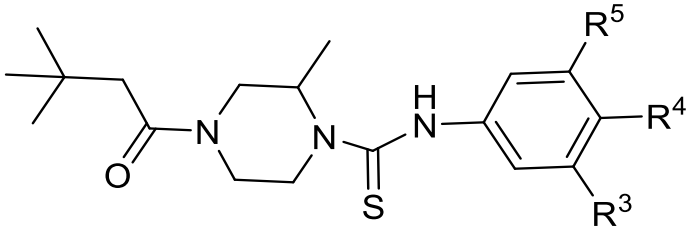

- Keep the **methyl piperazine ring** and the **phenylaminothiocarbonyl group**
- **Two points of structural variation:**
  - The **acyl group** at N-4 (**$R^1$**)
  - Substituents **$R^3$-$R^5$** on the **phenyl group**

# Methyl-piperazine derivatives
# Chemical Families description I

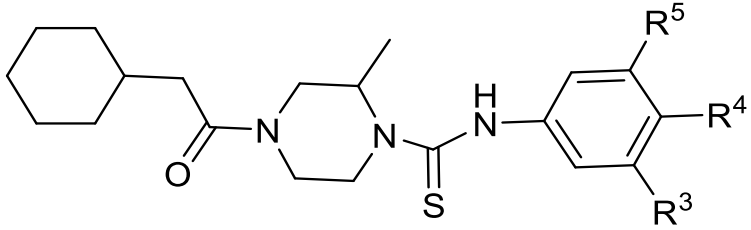
**General structure 1st family**



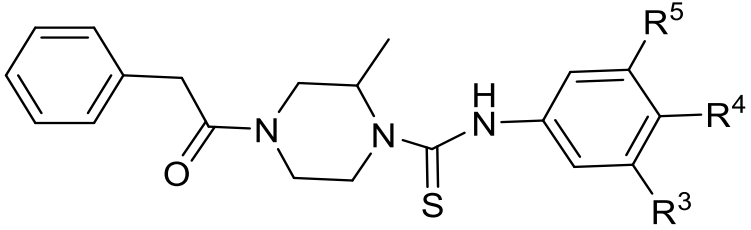

**General structure 2nd family**




**General structure 3rd family**

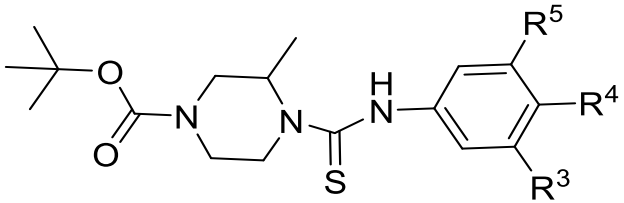



**General structure 4th family**



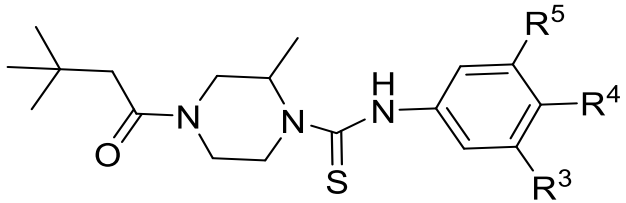
# Methyl-piperazine derivatives
# Chemical Families description II

**General structure 1st family**




| $R^3$ | $R^4$ | $R^5$ |
|---|---|---|
| - | $NO_2$ | - |
| - | Cl | - |
| - | CN | - |
| - | F | - |
| - | $CF_3$ | - |
| - | $OCH_3$ | - |
| - | $CH_3$ | - |
| $CF_3$ | - | $CF_3$ |

**General structure 2nd family**

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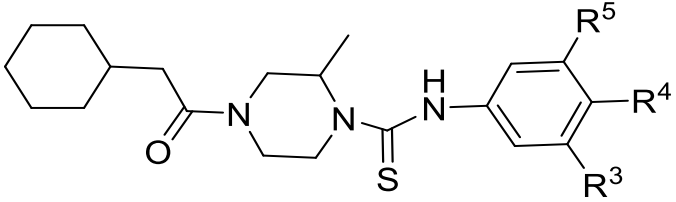
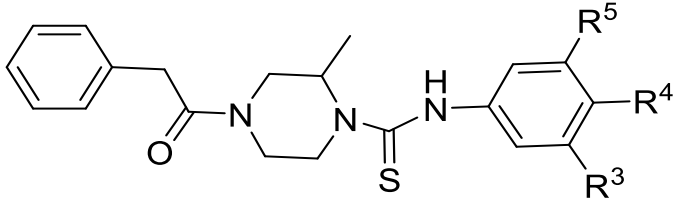


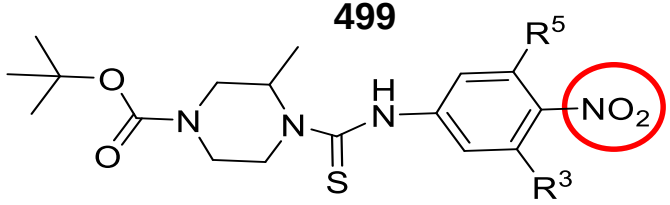

**General structure 4th family**



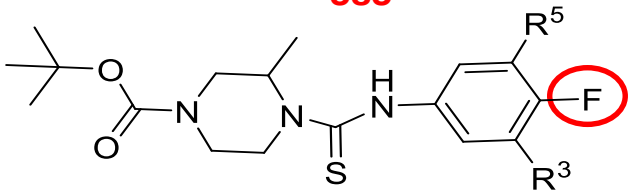
# Methyl-piperazine derivatives
# Antiviral activity I

| COMPOUND | $IC_{50}$ (μM) | YIELD REDUCTION (fold-reduction) | $CC_{50}$ |
|---|---|---|---|
| **585** | 1.78 ± 0.87 | 12.08 ± 2.78 | 200.00 |
| **613** | 2.53 ± 0.77 | 9.30 ± 2.90 | 193.04 |
| **616** | 0.57 ± 0.23 | 30.53 ± 12.94 | 143.36 |
| **619** | 2.06 ± 0.42 | 39.05 ± 15.95 | 122.21 |
| **628** | 2.04 ± 0.39 | 25.59 ± 10.45 | 210.38 |
| **634** | 5.12 ± 0.47 | 33.42 ± 10.16 | 129.74 |
| **635** | 4.59 ± 0.10 | 18.39 ± 5.44 | 174.69 |

**499**




**585**



# Methyl-piperazine derivatives
# Antiviral activity II

| COMPOUND | $IC_{50}$ (μM) | YIELD REDUCTION (fold-reduction) | $CC_{50}$ |
|---|---|---|---|
| **585** | 1.78 ± 0.87 | 12.08 ± 2.78 | 200.00 |
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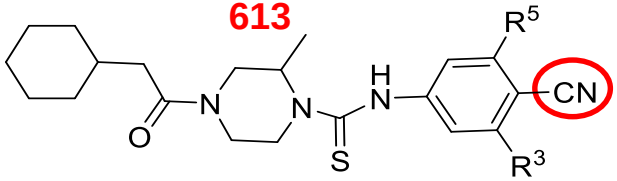
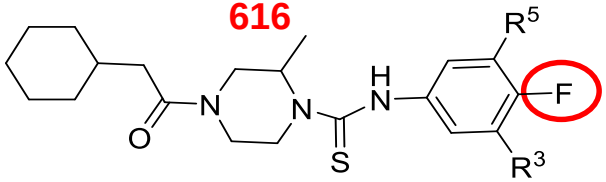
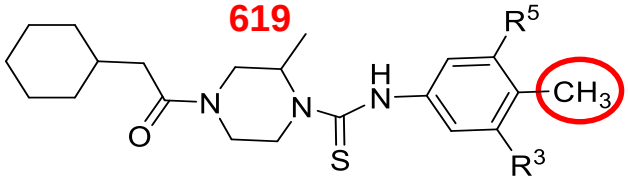
**613**




**616**



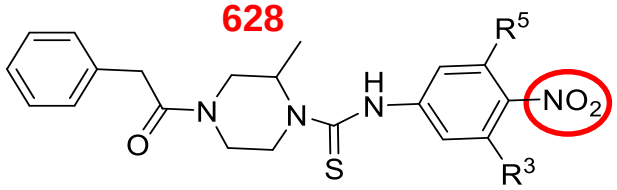

**619**



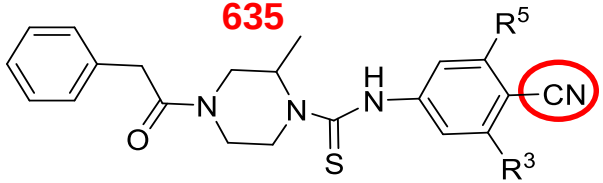
# Methyl-piperazine derivatives
# Antiviral activity III

| COMPOUND | $IC_{50}$ (μM) | YIELD REDUCTION (fold-reduction) | $CC_{50}$ |
|---|---|---|---|
| **585** | 1.78 ± 0.87 | 12.08 ± 2.78 | 200.00 |
| **613** | 2.53 ± 0.77 | 9.30 ± 2.90 | 193.04 |
| **616** | 0.57 ± 0.23 | 30.53 ± 12.94 | 143.36 |
| **619** | 2.06 ± 0.42 | 39.05 ± 15.95 | 122.21 |
| **628** | 2.04 ± 0.39 | 25.59 ± 10.45 | 210.38 |
| **634** | 5.12 ± 0.47 | 33.42 ± 10.16 | 129.74 |
| **635** | 4.59 ± 0.10 | 18.39 ± 5.44 | 174.69 |

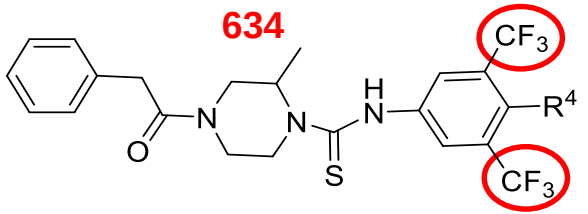
**628**



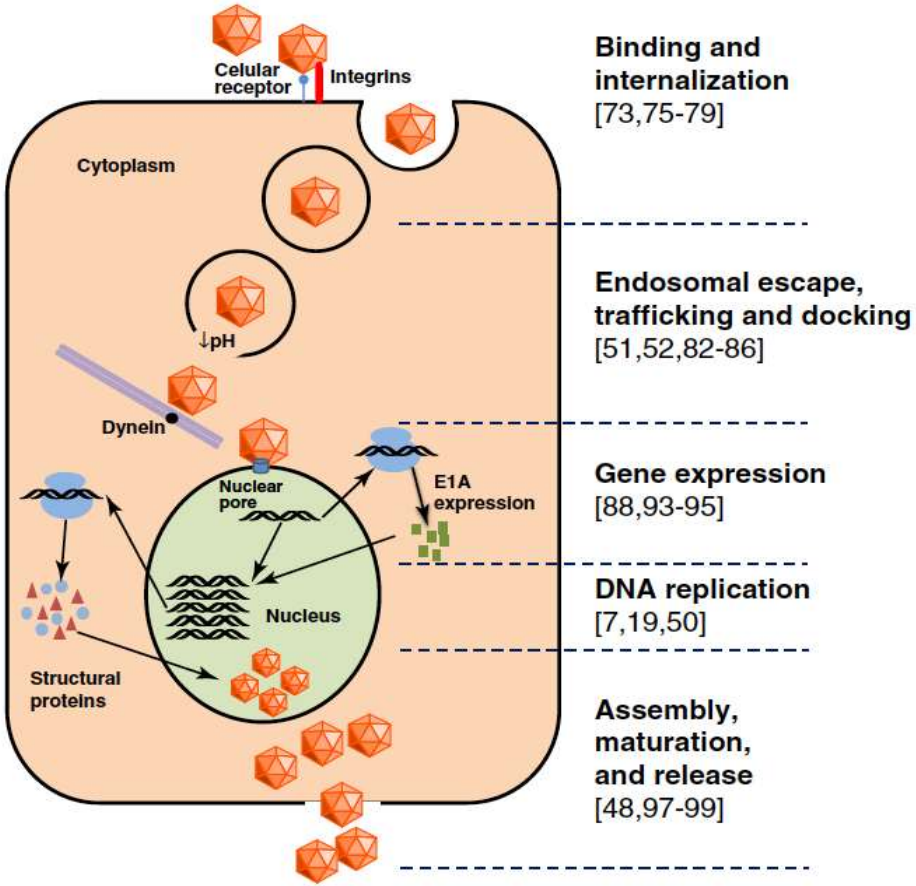

**635**




**634**



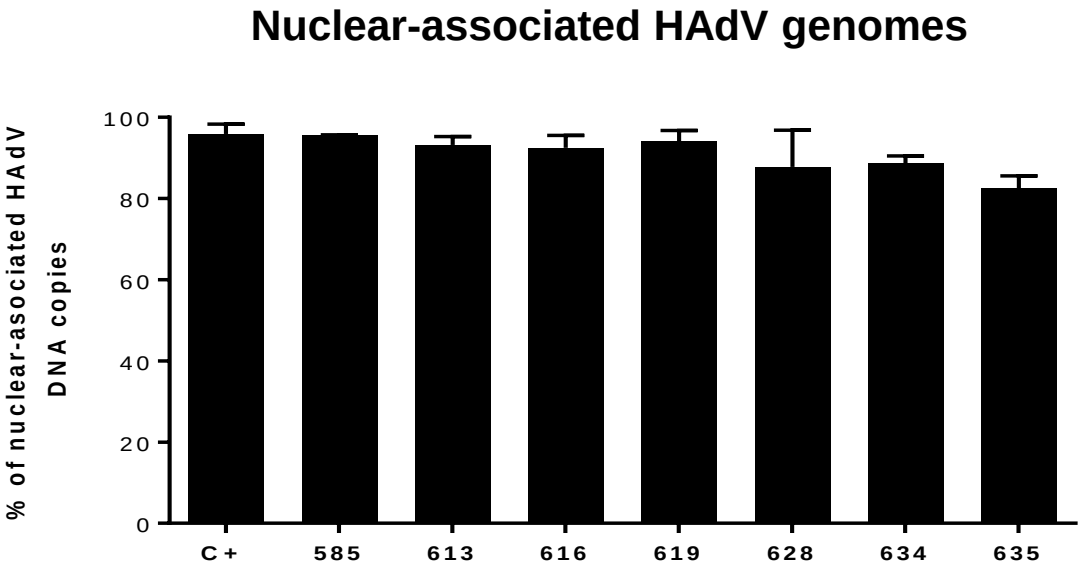
# Methyl-piperazine derivatives
# HAdV replicative cycle




From: **Martínez –Aguado et al., Drug Discov Today. 2015 Oct;20(10):1235-42**

# Methyl-piperazine derivatives Mechanistics I

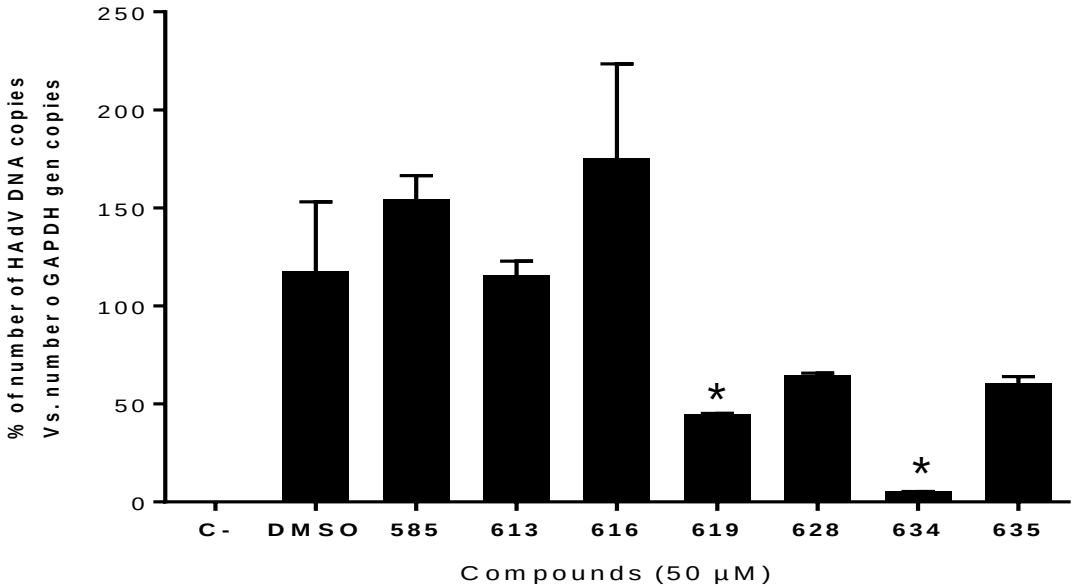
Any of these 7 **thiourea derivatives** showed a significant inhibition of HAdV entry




| Compound | Nuclear-associated HAdV genomes (%) |
|---|---|
| **585** | 95.54 ± 0.17 |
| **613** | 93.22 ± 2.01 |
| **616** | 92.51 ± 3.07 |
| **619** | 94.04 ± 2.73 |
| **628** | 87.76 ± 9.09 |
| **634** | 88.72 ± 1.76 |
| **635** | 82.49 ± 3.05 |

# Methyl-piperazine derivatives Mechanistics II

Compounds **619**, **628**, **634** and **635** inhibit significantly the *de **novo*** **synthesis of HAdV DNA** at 24h post-infection

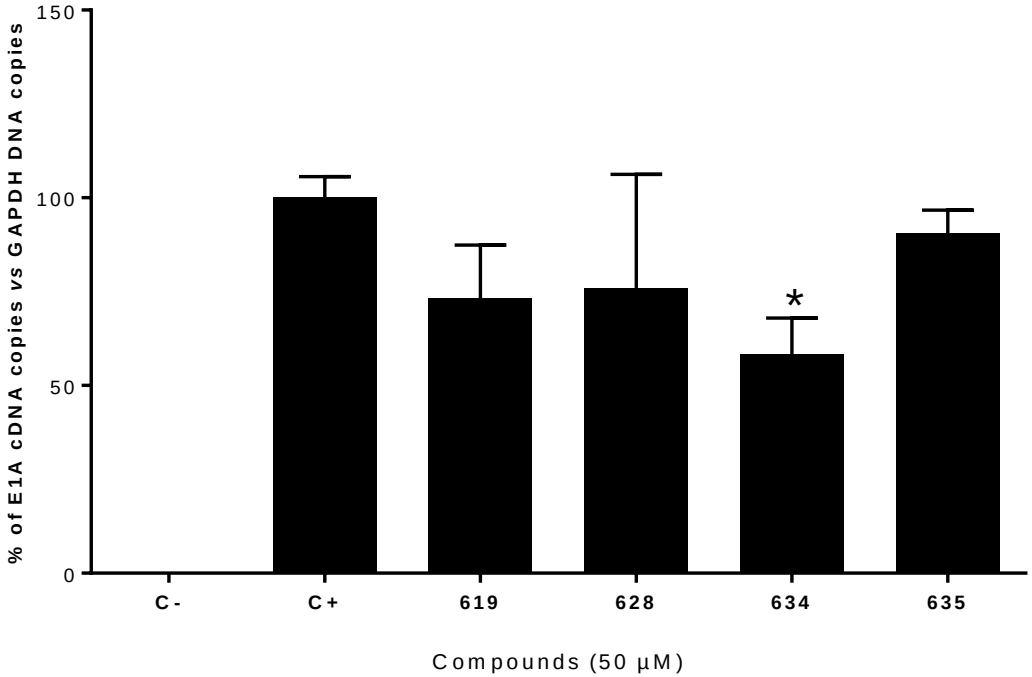



| Compound | DNA synthesis (%) |
|---|---|
| **585** | 154.10 ± 7.32 |
| **613** | 115.57 ± 48.15 |
| **616** | 175.28 ± 0.22 |
| **619** | 44.88 ± 1.09 |
| **628** | 64.67 ± 0.08 |
| **634** | 5.22 ± 3.33 |
| **635** | 60.55 ± 3.33 |

*ANOVA, Dunnett's test; $p < 0.05$

# Methyl-piperazine derivatives Mechanistics III

Compounds **634** significantly reduced the **mRNA copy number of E1A** gene at 6 h post-infection




| Compound | mRNA synthesis (%) |
|---|---|
| **619** | 73.23 ± 14.79 |
| **628** | 73.95 ± 30.28 |
| **634** | 58.35 ± 9.63 |
| **635** | 90.41 ± 6.31 |

*ANOVA, Dunnett's test; $p < 0.05$

# Conclusions

1. **4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives** have demonstrated to be a great source of molecules with **anti-HAdV activity**
2. Molecules **585**, **613**, **616**, **619**, **628**, **634** and **635** presented significant **anti-HAdV activity** at **low micromolar** concentration **without** significant **cytotoxicity**
3. **Any of these** 7 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives **blocked HAdV infection during the entry steps**
4. Compounds **619, 628, 635** and **634** showed a significant inhibition of the HAdV DNA replication process
5. Compound **634** has proven to be a significant inhibitor of HAdV infection **targeting transcription** of the immediately early gene E1A
6. Compounds **585**, **613** and **616** seem to be targeting a later step on the HAdV replicative cycle after HAdV DNA replication

# Conclusions

1. **4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives** have demonstrated to be a great source of molecules with **anti-HAdV activity**
2. Molecules **585**, **613**, **616**, **619**, **628**, **634** and **635** presented a significant **anti-HAdV activity** at **low micromolar** concentration **without** significant **cytotoxicity**
3. **Any of these** 7 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives **blocked HAdV infection during the entry steps**
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5. Compound **634** has proven to be a significant inhibitor of HAdV infection **targeting transcription** of the immediately early gene E1A
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# Conclusions

1. **4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives** have demonstrated to be a great source of molecules with **anti-HAdV activity**
2. Molecules **585**, **613**, **616**, **619**, **628**, **634** and **635** presented a significant **anti-HAdV activity** at **low micromolar** concentration **without a** significant **cytotoxicity**
3. **Any of these** 7 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives **blocked HAdV infection during the entry steps**
4. Compounds **619, 628, 635** and **634** showed a significant inhibition of the HAdV DNA replication process
5. Compound **634** has proven to be a significant inhibitor of HAdV infection **targeting transcription** of the immediately early gene E1A
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# Conclusions

1. **4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives** have demonstrated to be a great source of molecules with **anti-HAdV activity**
2. Molecules **585**, **613**, **616**, **619**, **628**, **634** and **635** presented a significant **anti-HAdV activity** at **low micromolar** concentration **without** significant **cytotoxicity**
3. **Any of these** 7 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives **blocked HAdV infection during the entry steps**
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5. Compound **634** has proven to be a significant inhibitor of HAdV infection **targeting transcription** of the immediately early gene E1A
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# Conclusions

1. **4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives** have demonstrated to be a great source of molecules with **anti-HAdV activity**
2. Molecules **585**, **613**, **616**, **619**, **628**, **634** and **635** presented a significant **anti-HAdV activity** at **low micromolar** concentration **without** significant **cytotoxicity**
3. **Any of these** 7 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives **blocked HAdV infection during the entry steps**
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5. Compound **634** has proven to be a significant inhibitor of HAdV infection **targeting transcription** of the immediately early gene E1A
6. Compounds **585**, **613** and **616** seem to be targeting a later step on the HAdV replicative cycle after HAdV DNA replication

# Conclusions

1. **4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives** have demonstrated to be a great source of molecules with **anti-HAdV activity**
2. Molecules **585**, **613**, **616**, **619**, **628**, **634** and **635** presented a significant **anti-HAdV activity** at **low micromolar** concentration **without** significant **cytotoxicity**
3. **Any of these** 7 4-acyl-1-phenylaminothiocarbonyl-2-methylpiperazine derivatives **blocked HAdV infection during the entry steps**
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6. Compounds **585**, **613** and **616** seem to be targeting a later step on the HAdV replicative cycle after HAdV DNA replication

# Acknowledgements


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**University of Seville**

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**Prof. José Manuel Vega Pérez, BSc, PhD**

**Margarita Vega Holm, BSc, PhD**

**Sarah Mazzotta, BSc**

AdenoNet

**Spanish Adenovirus Network: from basic biology to nanobiomedicine**

"Una manera de hacer Europa"