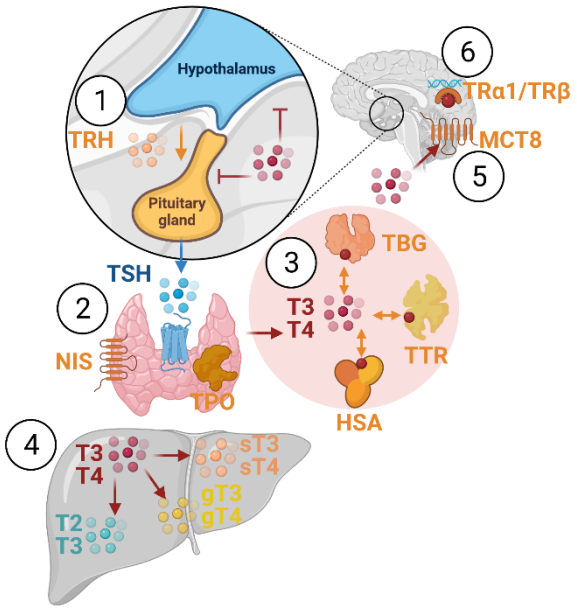
European Commission

STUDY REPORT

for the thyroperoxidase activity assay with Amplex UltraRed (AUR-TPO) – Part 2

EURL ECVAM validation study of a battery of mechanistic methods relevant for the detection of chemicals that can disrupt the thyroid hormone system



Nydahl K.

2023

Joint Research Centre

This study report has been prepared within the context of a collaboration agreement with the Joint Research Centre (JRC) Directorate for Health, Consumers and Reference Materials (Chemicals Safety and Alternative Methods Unit F3 / EURL ECVAM), for the validation of mechanistic methods to identify potential modulators of thyroid hormone signalling. It aims to provide evidence-based scientific support to the European policymaking process. The contents of this publication do not necessarily reflect the position or opinion of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use that might be made of this publication. For information on the methodology and quality underlying the data used in this publication for which the source is neither Eurostat nor other Commission services, users should contact the referenced source. The designations employed and the presentation of material on the maps do not imply the expression of any opinion whatsoever on the part of the European Union concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

This study report describes the experimental design and includes data generated in Part 2 of the validation study. The method was developed by US EPA and subsequently implemented by the EU-NETVAL test facility RISE (Sweden) within the validation study.

Contact information

EU-NETVAL facility
RISE Research Institutes of Sweden AB
Sara Bogren, Facility Manager GLP
Box 857, 501 15, Borås, Sweden
Arvid Wallgrens Backe 20 | SE-413 46 Göteborg
M: +46 70 915 18 08
sara.bogren@ri.se
https://www.ri.se/en

EU Science Hub
https://joint-research-centre.ec.europa.eu

JRC133077

Ispra: European Commission, 2023

How to cite this report: Nydahl K., Study report for the thyroperoxidase activity assay with Amplex UltraRed (AUR-TPO) – Part 2 of the EURL ECVAM thyroid validation study, European Commission, Ispra, 2023, JRC133077.

RI. SE

REPORT

Contact person RISE
Karin Nydahl
Division Materials and Production
+46 10 516 59 28
karin.nydahl@ri.se

Sponsor: EURL ECVAM
Att: Prof. Maurice Whelan,
European Commission,
JRC Directorate F - Health, Consumers and Reference Materials
Building 101, T.P. 126, office 02/2114,
Via E. Fermi 2749, Ispra, Italia

Report for assignment: Relevance assessment of the Thyroperoxidase Activity Assay with Amplex UltraRed (AUR-TPO)

(4 appendices)

Purpose and applicability

The purpose of this study was to assess the relevance of the AUR-TPO *in vitro* method by generating data in at least three independent valid runs for 30 test items provided by the sponsor, together with reference and control items. The method is described in SOP RISE 5581 v 2.0 "Thyroperoxidase activity assay with Amplex UltraRed (AUR-TPO)". The method was developed for the detection of compounds with ability to inhibit the enzyme thyroperoxidase, thus acting as thyroid disruptors.

Study ID: 8P06603:B

Assignment

Generation of experimental data for 30 test items according to the AUR-TPO *in vitro* method described in SOP RISE 5581 v 2.0 "Thyroperoxidase activity assay with Amplex UltraRed (AUR-TPO)". The testing was further specified at RISE in standard operating procedures SOP RISE 5519 v 3.0 "Culture of FTC 238 and FTC 238/hrTPO cells", SOP RISE 5582 v 1.0 "Thyroperoxidase (TPO) extract preparation", and SOP RISE 5569 v 2.0 "Solubility determination by visual inspection".

Test description

Cellular extracts of recombinant FTC-238 cells overexpressing human thyroperoxidase (TPO) are exposed to the test items in presence of the fluorogenic substrate Amplex® UltraRed (AUR) and an excess of hydrogen peroxide. Functional thyroperoxidase converts Amplex® UltraRed to fluorescent Amplex® Ultroxred, which can be measured with a fluorimeter. Test items with an endocrine-disruptive effect, able to impair thyroperoxidase function, will give rise to a decrease in signal in the assay. In addition, test items that show an effect in the first assay are further evaluated in a separate control assay utilising recombinant luciferin and measuring light emitted by luciferase. The purpose of the control assay is to verify that the inhibition shown in the AUR-TPO assay is specific to thyroperoxidase and not a general enzyme-inhibiting effect.

Study dates

Study initiation date: 2021-11-01
Experimental starting date: 2021-12-03
Experimental completion date: 2022-09-27

Test items

Information regarding the test items is summarized in Table 1.

Table 1. Test item details.

RISE ID	EURL ECVAM Chemical code	State, storage	Approximate molecular weight (Da)	Amount received	Hazard label
A427	427	Solid RT	250	316 mg	H302
B258	258	Liquid RT	Aqueous solution (1 M)	1 mL	H302+H332, H314, H351, H360, H362, H372, H411
C700	700	Solid RT	350	526 mg	H317, H319, H400
D322	322	Solid -20°C	350	2 x 25mg original vials	None
E073	073	Solid RT	175	323 mg	H302, H351
F808	808	Solid 4°C	500	3 x 10mg original vials	H302
G777	777	Solid RT	125	319 mg	H271, H302, H319, H373
H083	083	Solid RT	250	313 mg	H302, H317, H319
I488	488	Solid RT	550	310 mg	H410
J171	171	Liquid RT	300	1mL	H360FD, H410
K047	047	Solid RT	200	333 mg	H302
L465	465	Solid RT	275	306 mg	H301+H311, H315, H319, H330, H335, H351, H410
M192	192	Solid RT	300	329 mg	H315, H319, H410
N356	356	Solid 4°C	350	318 mg	H315, H317, H319, H334
O257	257	Solid 4°C	150	320 mg	H302
P137	137	Solid RT	125	328 mg	H302, H351, H360D, H372

RISE ID	EURL ECVAM Chemical code	State, storage	Approximate molecular weight (Da)	Amount received	Hazard label
Q315	315	Solid RT	200	333 mg	H302+H332, H318, H335, H341, H361d, H372, H411
R498	498	Solid RT	325	337 mg	H301, H361d, H372, H411
S074	074	Solid 4°C	325	313 mg	H302, H315, H317, H319, H334, H335
T879	879	Solid 4°C	700	324 mg	H315, H319, H361fd, H362, H373
U778	778	Solid 4°C	275	311 mg	None
V050	050	Solid RT	275	339 mg	H315, H319
W796	796	Solid -20°C	750	273 mg	H300 Fatal if swallowed
X573	573	Solid 4°C	550	312 mg	H301, H360F, H373, H400, H410
AA039	039	Solid RT inert gas	300	311 mg	H301+H311+H331, H315, H319
AB253	253	Solid -20°C	475	313 mg	None
AC426	426	Solid RT inert gas	200	316 mg	H301, H330, H340, H350, H360fd, H372, H410
AD060	060	Solid RT	175	317 mg	H317, H410
AE098	098	Solid RT	125	328 mg	H302, H315, H318, H400
AF364	364	Solid RT inert gas	375	313 mg	None

Date of test items arrival at RISE: 2021-10-26

Disposal of test items: Test items will be kept until the completion of the study. Thereafter all materials will be destroyed unless return is requested by the sponsor.

Materials

Reference and control items

Reference item for the AUR-TPO *in vitro* method is 2-mercapto-1-methylimidazole (MMI). Positive control for the assay is 6-propyl-2-thiouracil (PTU) and negative control is 2-hydroxy-4-methoxybenzophenone (BP3). Reference item for the control assay Quantilum inhibition (QLI) is luciferase inhibitor II (Lucinh2). Positive control for the QLI control assay is luciferase inhibitor I (Lucinh1) and negative control is 2-hydroxy-4-methoxybenzophenone (BP3). The chemicals are provided by the sponsor, except Lucinh1 and Lucinh2 which were purchased by RISE. Details regarding the chemicals are listed in Table 2.

Table 2. Reference and control item details.

Chemical name	Lot /batch number	Purity	Storage	Expiry date	CAS number	Hazard label
BP3	WXBC6458V	99.8 %	RT	No exp date – responsibility of sponsor	131-57-7	H315, H319, H335
LUCINH1	3703240, 3810523	98.1% (both batches)	2-8°C	Retest date 21 Oct 2027 (both batches)	352341-26-5	None
LUCINH2	3492236, 3574625	97.7% (both batches)	2-8°C	Retest date 07 Nov 2023 (both batches)	10205-56-8	None
MMI	WXBC8588V	99.7 %	RT	No exp date – responsibility of sponsor	60-56-0	H317, H361
PTU	BCBR8708V	99.2 %	RT	No exp date – responsibility of sponsor	51-52-5	H302, H351

Reagents

- Amplex UltraRed reagent (Thermo Fisher, cat # A36006)
- KH_2PO_4, CAS 7778-77-0, Potassium phosphate monobasic, 99% (Sigma (Merck) cat # P5379)
- K_2HPO_4, CAS 7758-11-4, Potassium phosphate dibasic, 98% (Sigma (Merck) cat # P3786)
- DMSO, CAS 67-68-5, Anhydrous dimethyl sulfoxide (Sigma (Merck) cat # 276855)
- Ammonia solution 25%, CAS 1336-21-6 (Sigma (Merck) cat # 1.05432)
- Hydrogen peroxide solution, 30% (w/w) in H_2O, CAS 7722-84-1 (Sigma (Merck) cat # H1009)
- Sodium deoxycholate, CAS 302-95-4 (Thermo Fisher Scientific, cat # 89904)
- Luciferase assay reagent (Promega, cat # E1501)
- QuantiLum® Recombinant Luciferase (Promega, cat # E170)
- Bovine serum albumin (BSA) (GE Healthcare Life Sciences Hyclone Laboratories, cat # SH30574.01)
- Deionized water

For preparation of TPO extracts:

- Iscove's modified Dulbecco's medium (1×) buffered with $NaHCO_3$ (Gibco Life Technologies, cat # 21056-023)
- Fetal Bovine Serum (Gibco Life Technologies, cat # 10270-098)

- Penicillin-streptomycin (Cytiva Hyclone, cat # SV30010)
- Geneticin (G-418 sulfate) (Gibco Life Technologies, cat # 10131-035)
- TrypLE-EDTA (Gibco Life Technologies, cat # A12177 and 15040033)
- DPBS without Ca_{2+}, Mg_{2+} (GE Healthcare Hyclone, cat # SH30028.02)
- Cell culture grade Dimethyl sulfoxide (DMSO) (Sigma Aldrich, cat # D2650) (for cryopreservation of cells)
- Hematin (Sigma Aldrich, cat# H3281)
- Cell culture grade water (e.g. Cytiva Hyclone, cat # SH30529.02)
- Sodium hydroxide solution to dissolve hematin (Merck KGaA, cat # 1.09959.0001)
- Bovine serum albumin (BSA) standard (Thermo Scientific, cat # 23209)
- Pierce™ BCA Protein Assay Kit (Thermo Scientific, cat # 23225 and 23227)

Important equipment and disposables

- 96-well compound storage plates, Corning® 96 Well Storage Microplates, Corning Costar cat # 3365, for long-term storage of DMSO stock solutions and efficient preparation of dilution series, equipped with sealing mat (Corning Costar, cat # 3080).
- Black solid 96-well plates (Corning Costar, cat # 3356) (AUR-TPO assay)
- White solid 96-well plates (Corning Costar, cat # 3912) (QLI assay)
- Luminometer/Absorbance/Fluorescence plate reader Synergy 2 SLFAD (plate reader with dual dispense modules), BioTek Instruments Inc.

Test system description

Test system description

The test system in this assay is a whole cell extract of recombinant follicular thyroid carcinoma cells (FTC-238) expressing human TPO. In the implementation of the study, a whole cell extract of the wildtype cells was evaluated and found to have no activity in the assay, hence the activity measured is attributable to the recombinant TPO protein. The cells used to produce the test system were originally constructed by Prof J. Köhrle at Charité, Berlin, using the vector pCDNA3.1 with G418 resistance gene and human thyroid peroxidase as insert and transfected using Lipofectamine Plus (Invitrogen). The cells were provided to the RISE test facility by the sponsor. The sponsor has characterised the cells as being free from cross-contamination with mouse, rat, Syrian hamster, or Chinese hamster, cells; to be free from mycoplasma; to be free from HIV-1, HIV-2, Hepatitis B, and Hepatitis C, virus; and to have an STR profile matching the original wildtype cell line. At RISE, the cells have been expanded to master and working cells banks that each have been confirmed to be free from mycoplasma and free from other contaminations. The cell culture was performed according to SOP RISE 5519 v 3.0 "Culture of FTC-238 and FTC-238/hrTPO cells". Whole cell extracts were prepared according to SOP RISE 5582 v 1.0 "Thyroperoxidase (TPO) extract preparation" in several batches that were being stored at -80°C. The cells were stored at -150 °C and cultured at 37 °C, 5% CO_2 in a humidified incubator. Extracts were made from cells in passage number 7-11. When first used in experiments, each batch of extracts was verified to meet the acceptance criteria in the assay ("TPO efficiency" > 3 and expected AC_{50} of reference item MMI). Further, the continued activity ("TPO efficiency") of the TPO protein was verified to meet the acceptance criteria on each plate in each experimental round.

Test system management

Aliquots of whole cell extracts were stored at -80 °C and were thawed on ice for each experimental round of the assay. The test system was kept cold for as long as possible during the assay, and the work was performed swiftly after that the test system could no longer be kept on ice.

All work with cells was performed in a biological safety cabinet class II inside an ISO class 7 environment using aseptic techniques. Work with cells was required for additional production of TPO test system during the study.

Test system quality control

After completion of the study, one vial from the working cell bank of TPO-transfected cells (FTC-238/hrTPO) used to generate TPO extracts, as well as one vial for each batch of TPO extract prepared in the study are stored in freezer, and ready to be sent to the sponsor upon request. Quality control data is presented in Appendix 3.

Method

The evaluation of the endocrine disrupting capacity of the test items was performed according to SOP RISE 5581 v 2.0. The testing procedure is briefly outlined below.

Test procedure

Before the start of the test, the maximum solubility of the test items was determined according to SOP RISE 5569 v 2.0. The highest concentration evaluated was 100 mM for all test items. The test chemicals were dissolved in appropriate solvent and examined for signs of particles by visual inspection. Then the solutions were centrifuged and vials were checked for deposited precipitates. The procedure was repeated for dilutions in buffer. The obtained maximum solubility is presented in the Results section.

The whole cell extract of cells overexpressing thyroperoxidase was stored at -80 °C and was thawed on ice for each experimental round of the assay. At the start of the test, 12.5 ng of protein per well was added to a black 96 well plate. Test items/controls were added, followed by the Amplex® UltraRed reagent and the reaction was then initiated by adding an excess of H_2O_2. The plate layouts are presented in Appendix 2. The plate was incubated for 30 minutes at 37 °C before measuring fluorescence at ex/em wavelengths 540/600 nm with dichroic mirror 570 nm.

For the AUR-TPO assay, range-finding experiments were carried out to:

1) Confirm that the test item was soluble in both stock and work solutions at certain concentrations
2) Determine if the test item displayed inhibition of enzyme or not.
3) Select the concentration (C8, the highest concentration) and dilution factor that most likely would provide a full dose-response curve (for a test item showing a full or partial response).

An experiment was considered valid when all acceptance criteria outlined in SOP RISE 5581 v 2.0 had been met. For each experiment, test items were weighed out and dissolved independently.

Test items that did not show a response were continued to be tested with the range-finding dilution range 1:10. The data set was considered complete when two additional valid range-finding experiments had been performed.

In case the inhibition response was ≥ 20 % from solvent control in the range-finding assay, a main test was performed in the next experimental round to enable calculation of all dose-response curve parameters. Three valid main assay runs were obtained per test chemical showing inhibitory response.

Test items that showed inhibitory response were further evaluated in the specificity control assay, QuantiLum inhibition (QLI). 6 ng of recombinant Quantilum luciferase were added to each well of a white 96 well plate. Test items/controls were added, plate layouts are presented in Appendix 2. The plate was incubated for 30 minutes at 37 °C. Thereafter luciferin reagent (Luciferase assay system) was added to the plate one well at a time using one of the dispensers in the plate reader, and luminescence was detected for 1 second for each well immediately after dispensing.

Evaluation of Results

The analysis of the inhibitory effect was performed as described in SOP RISE 5581 v 2.0 using the Microsoft Excel data analysis forms exported from Gen5 protocols: "AUR_TPO_range_211209", "AUR_TPO_main_210201", "QLI_210603", and GraphPad Prism 9.4.1. The data analysis forms have inbuilt functions to check whether the acceptance criteria in SOP RISE 5581 v 2.0 are met:

AUR-TPO assay:

- TPO efficiency (ratio between VC and BC2): >3
- Reference item MMI AC_{50}: $8.2*10^{-9} – 1.4*10^{-6}$
- Inhibition (%) for PC PTU 25 µM: > 50
- Inhibition (%) for NC BP3 100 µM: < 10
- Z-factor for MMI C8: ≥ 0.5
- Plate dynamic range (ratio between VC and BC1): >2
- Standard deviation of Inhibition (%) for each replicate of vehicle control, blanks, reference, control or test items on each plate: ≤ 20%

QLI assay:

- Reference item LUCINH2 AC_{50}: $2.0*10^{-9} – 2.0*10^{-8}$ M
- Inhibition (%) for PC LUCINH1: > 80
- Inhibition (%) for NC BP3 10 µM: < 20
- Z-factor for LUCINH2 C8: ≥ 0.5
- Standard deviation of Inhibition (%) for each replicate of vehicle control, blank, reference, control or test items on each plate: ≤ 20%

Manually checked additional acceptance criteria were: Maximum two concentrations may be excluded from the test item or reference item dilution series, on basis of operator errors or other information (including requirement for standard deviation of triplicates).

Results

Tables 3 and 4 give an overview of all valid and invalid range-finding ("Range") and main ("Main") experiments, carried out for each test item, for the AUR-TPO assay and for the control assay QLI. Invalid runs are indicated in italics and the reason for invalidity is indicated in footnotes below the table in each case.

Table 3. Overview of all experimental runs with the AUR-TPO assay carried out in Study 2. Invalid runs are indicated in italics and explained below the table. Unusual occurrences in valid runs are also explained below the table.

Chemical name; RISE Test item ID	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
A427	*Range*[1]	Range	Range	Range				
B258	Range	Range	Range					
C700	*Range*[1]	*Range*[2]	*Range*[2]	Range	Range	Range	Range	
D322	Range	*Range*[2]	*Range*[2]	Range	Range			
E073	*Range*[1]	Range	Main	Main	Main			
F808	Range	Main	Main	Main				
G777	*Range*[1]	Range	*Range*[2]	*Range*[2]	Range[3]	Range	*Range*[4]	Range
H083	*Range*[1]	Range	Main	Main	Main			
I488	*Range*[1]	*Range*[2]	*Range*[2]	Range	Main	Main	Main	
J171	*Range*[1]	*Range*[2]	*Range*[2]	Range	Range	*Range*[4]	Range	
K047	*Range*[1]	*Range*[2]	*Range*[2]	Range	Range	Range		
L465	Range	*Main*[2]	Main	Main	Main[3]	Main		
M192	Range	*Main*[2]	Main	Main	Main	Main[3]	Main	
N356	Range	*Range*[4]	Range	Range				
O257	Range	Range	*Range*[5]	Range				
P137	Range	*Main*[2]	Main	Main	Main[3]	Main		
Q315	*Range*[5]	Range	Range	Range				
R498	Range	*Range*[5]	Range	Range				
S074	Range	Range	Range					
T879	Range	Range	Range					
U778	Range	Main	Main	Main				
V050	Range	Main	Main	Main				
W796	Range	Main	Main	*Main*[6]	Main			
X573	Range	Range	Range					
AA039	Range	Range	Range					
AB253	Range	Range	Range					
AC426	Range	Range	Range					
AD060	Range	Main	*Main*[5]	Main	Main			
AE098	Range	Main	Main	Main				
AF364	Range	Main	Main	Main				

1. NC too high
2. TPO efficiency too low
3. Passed acceptance criteria, but base line is shifted, not at zero-level.
4. Plate dynamic range too low
5. Standard deviation too high
6. Raw fluorescence value overflow

Table 4. Overview of all experimental runs with the QLI assay carried out in Study 2. Invalid runs are indicated in italics and explained below the table.

Chemical name; RISE Test item ID	Run 1	Run 2	Run 3	Run 4
E073	Main	Main	*Main*[1]	
F808	Main	*Main*[2]	Main	Main
H083	Main	*Main*[2]	Main	Main
I488	Main	Main	Main	
L465	Main	*Main*[2]	Main	Main
M192	Main	*Main*[2]	Main	Main
P137	Main	*Main*[2]	Main	Main
U778	Main	Main	Main	
V050	Main	Main	*Main*[1]	
W796	Main	Main	*Main*[1]	
AD060	Main	*Main*[2]	Main	Main
AE098	*Main*[2]	Main	Main	Main
AF364	*Main*[2]	Main	Main	*Main*[1]

1. NC too high, results will be used anyway because it is only a control assay and results confirms previous findings.
2. NC too high.

Reference and control items

The obtained dose-response curves for AUR-TPO reference item MMI are shown in Figure 1 and the dose-response curves obtained for QLI reference item Luciferase inhibitor II are shown in Figure 2. Graphs for calculated parameters (plate dynamic range, Z-factor, TPO efficiency, MMI AC_{50} and CV for the vehicle control) for the AUR-TPO assay are presented in Figures 3-7. The determined relative inhibition for negative and positive control items are presented in Figures 8-9. The corresponding data is presented in tables in Appendix 3. Graphs for calculated parameters (Z-factor, Luciferase inhibitor II AC_{50} and CV for the vehicle control) for the QLI control assay are presented in Figures 10-12. The determined relative inhibition for negative and positive control items are presented in Figures 13-14. The corresponding data is presented in tables in Appendix 4.

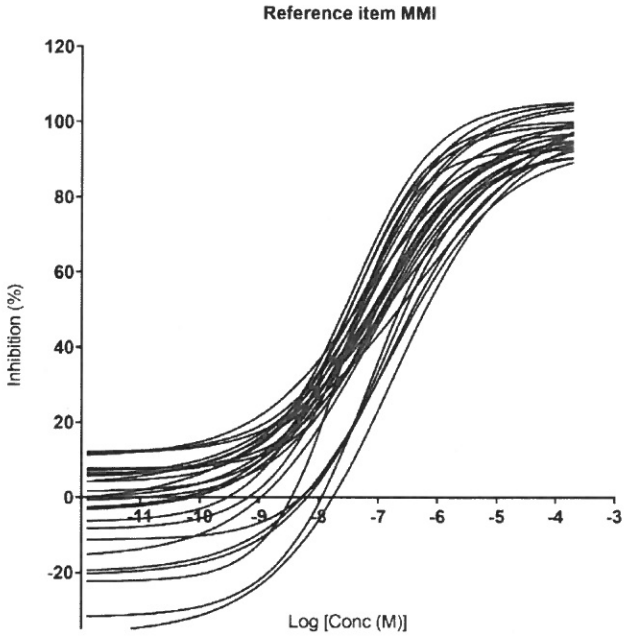


Figure 1. Obtained dose-response curves for AUR-TPO reference item MMI for all valid runs during the study.

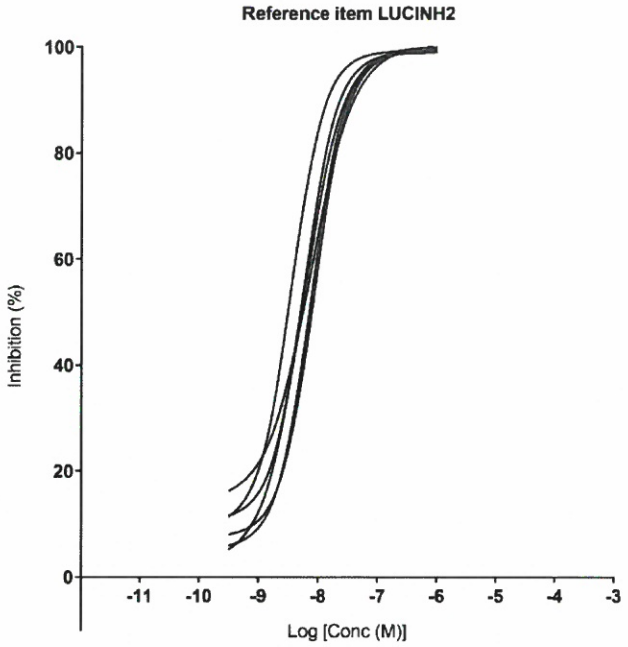


Figure 2. Obtained dose-response curves for QLI reference item luciferase inhibitor II.

Figure 3. TPO efficiency values for all plates in the AUR-TPO assay in the study.

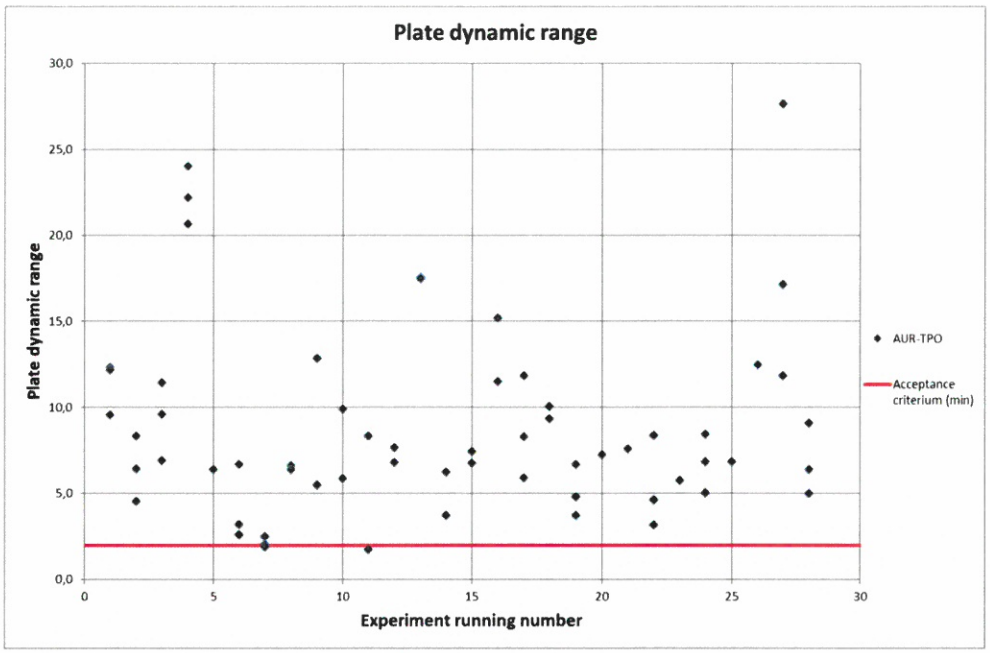


Figure 4. Plate dynamic ranges for all plates in the AUR-TPO assay in the study.

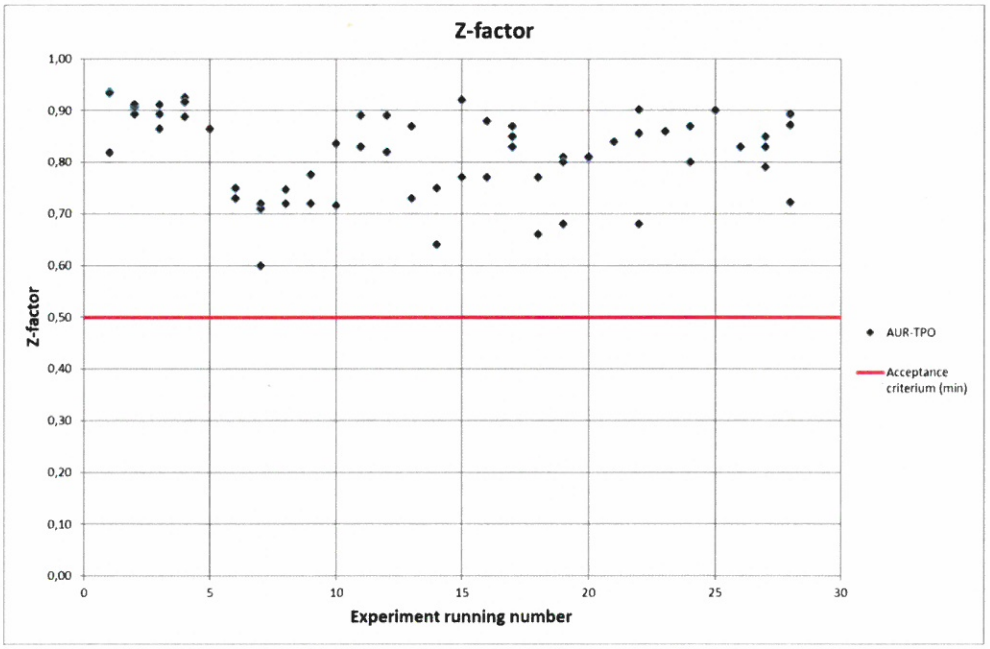


Figure 5. Z-factors for all plates in the AUR-TPO assay in the study.

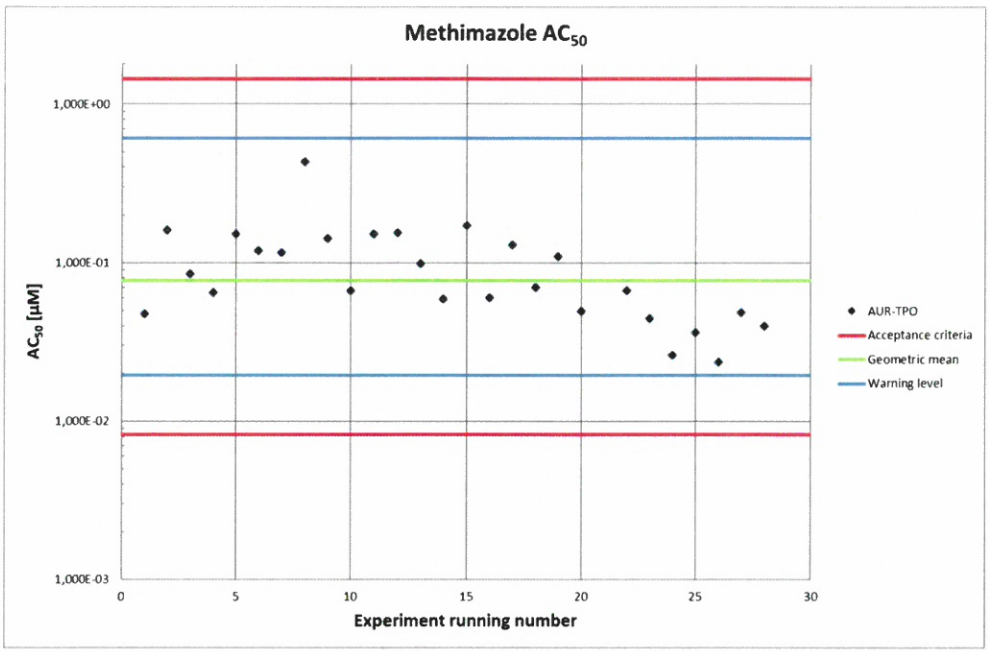


Figure 6. AC_{50} [µM] values for reference item MMI for the AUR-TPO assay in the study.

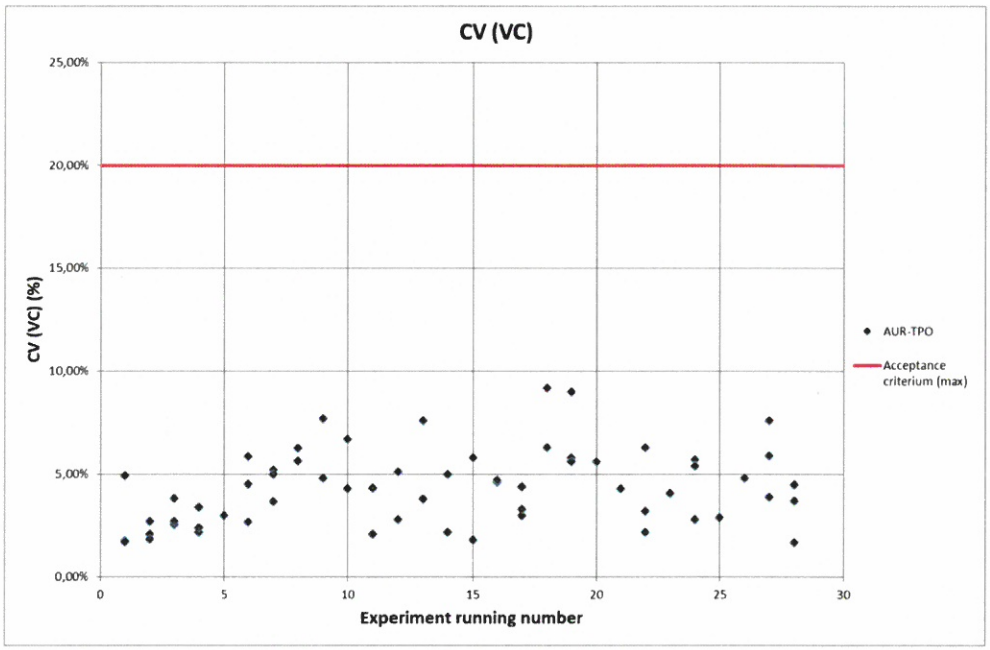


Figure 7. CV for the vehicle control (VC) for all plates in the AUR-TPO assay in the study.

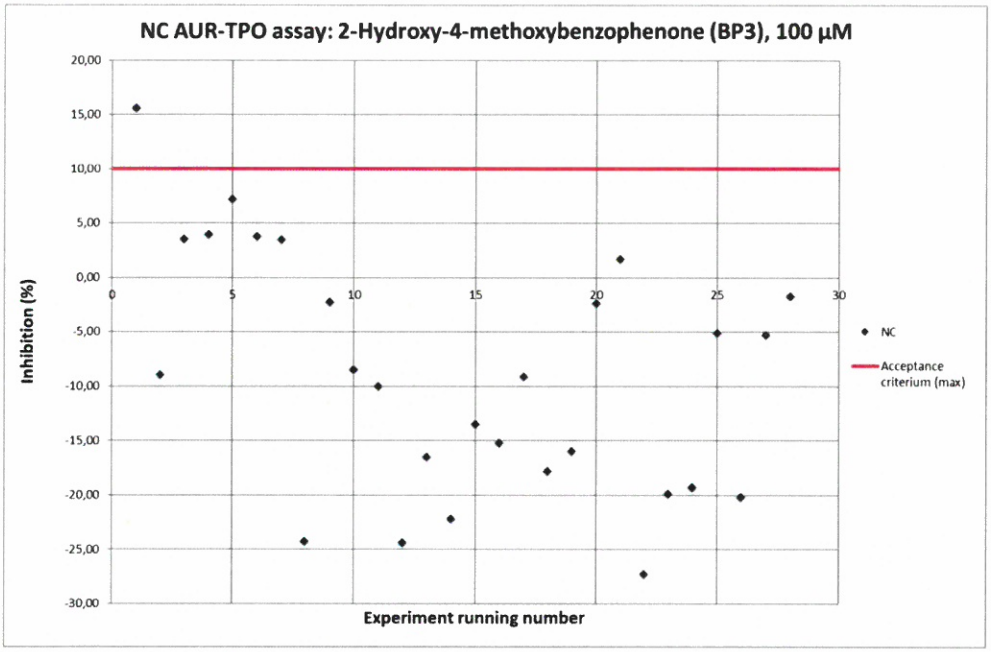


Figure 8. Relative inhibition (%) for negative control item BP3 in the AUR-TPO assay, for the runs in the study.

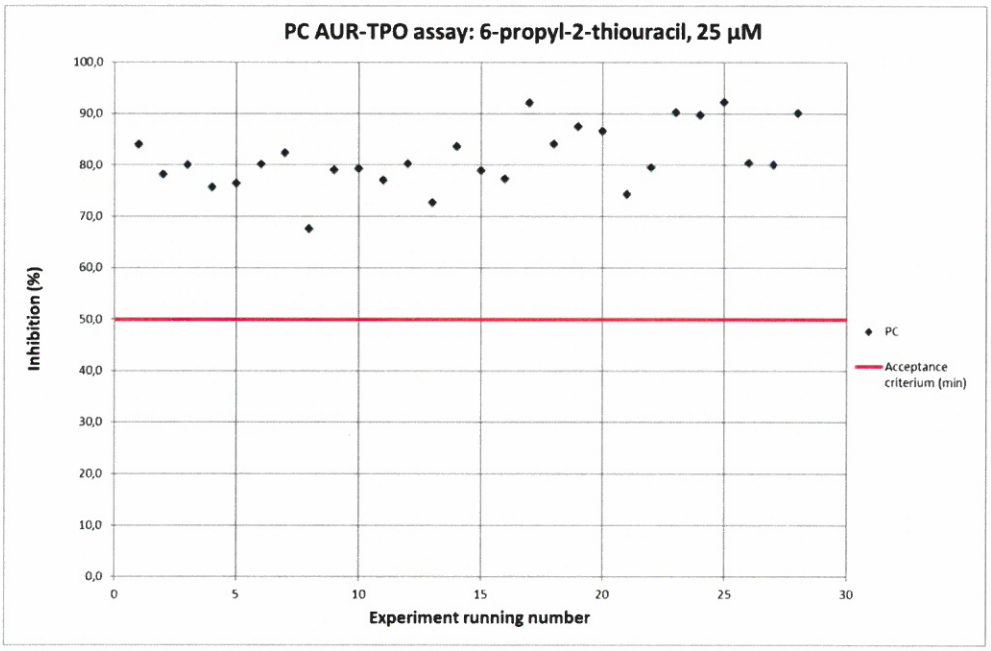


Figure 9. Relative inhibition (%) for positive control item PTU in the AUR-TPO assay, for the runs in the study.

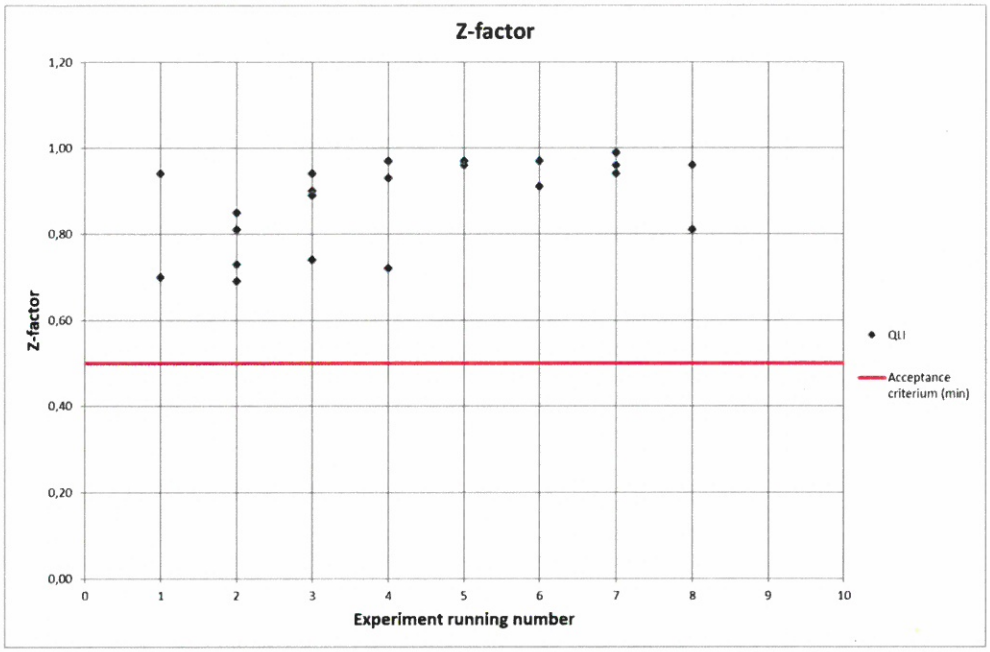


Figure 10. Z-factors for all plates in the QLI assay in the study.

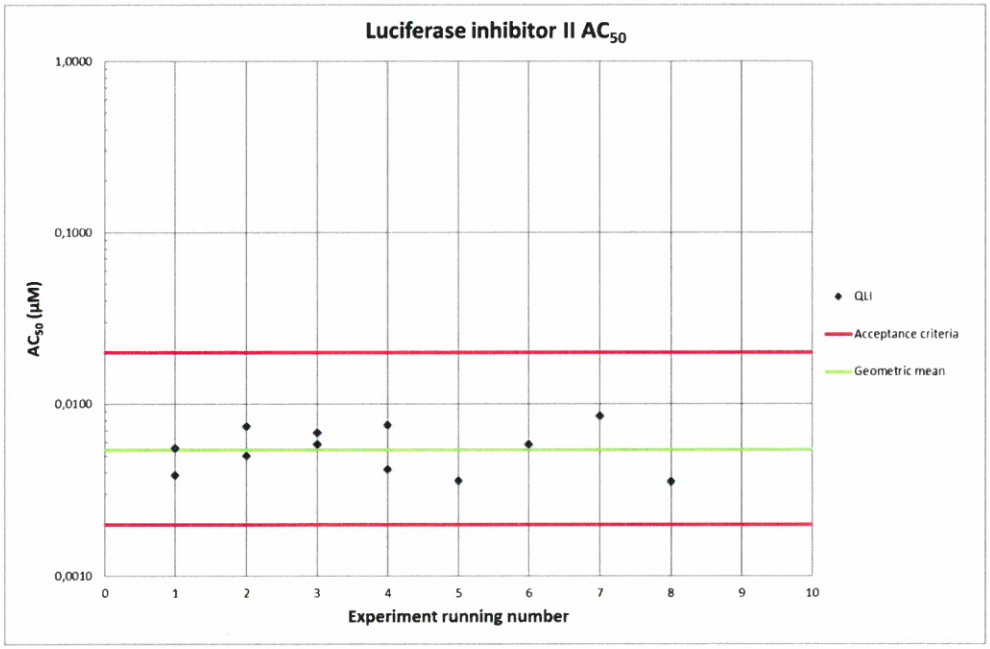


Figure 11. AC_{50} (µM) values for reference item Luciferase inhibitor II for the QLI assay in the study.

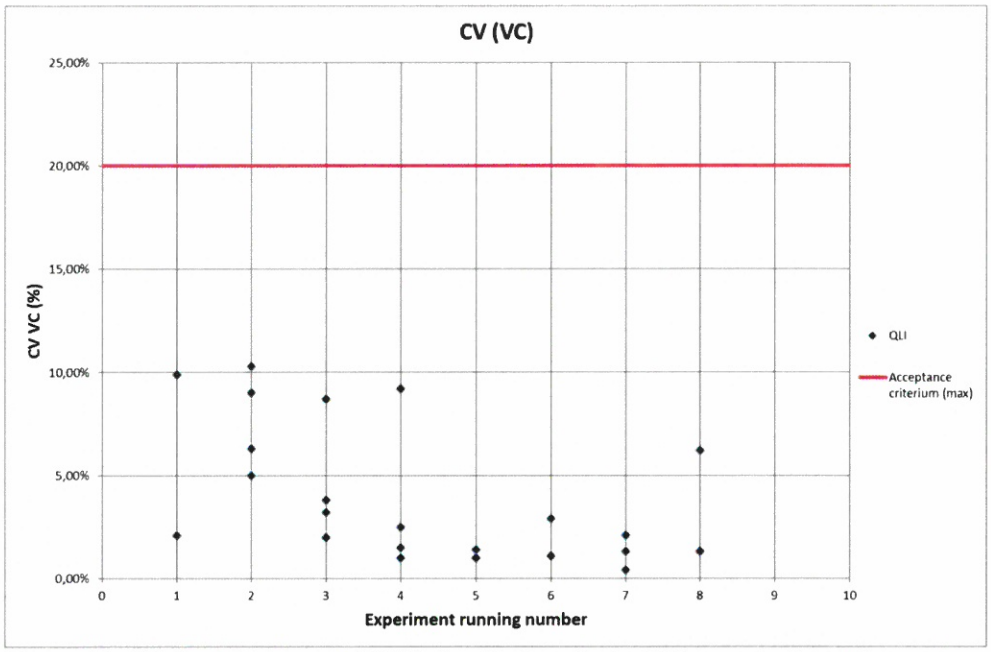


Figure 12. CV for vehicle control (VC) for all plates in the QLI assay in the study.

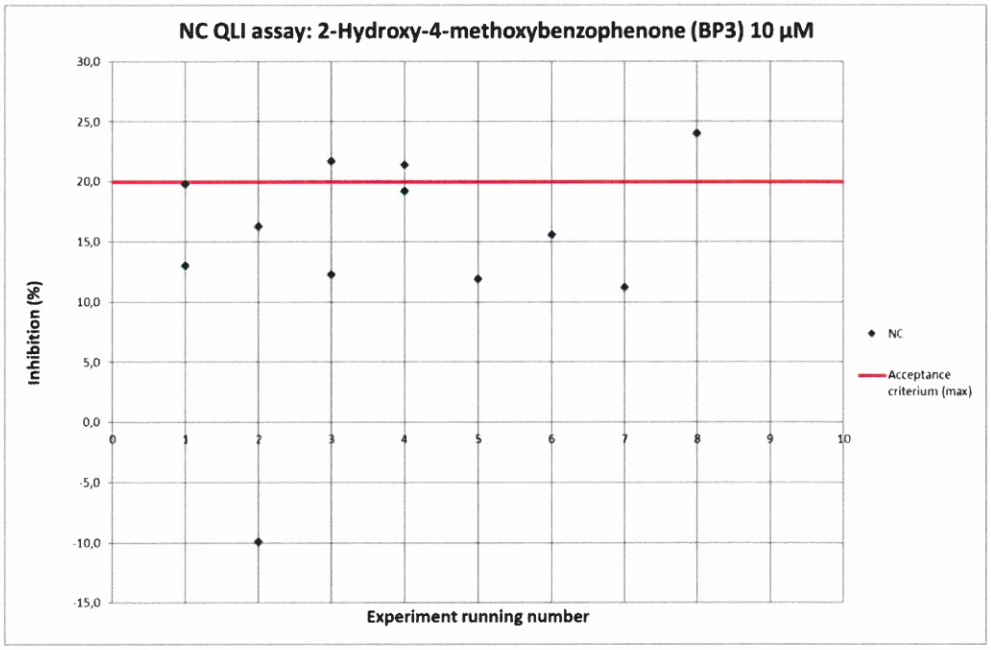


Figure 13. Relative inhibition (%) for negative control item BP3 in the QLI assay in the study.

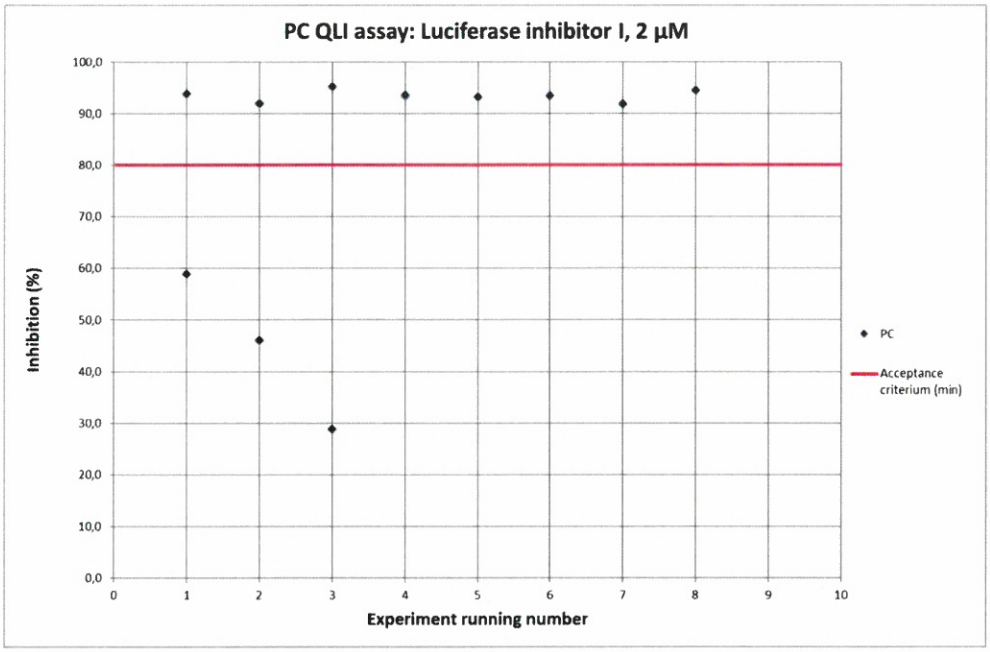


Figure 14. Relative inhibition (%) for positive control item Luciferase inhibitor I in the QLI assay in the study.

Test items

Solubility evaluation

All test items were dissolved in DMSO, except for item Q315. The procedure to obtain full solubility and the soluble concentrations in DMSO are given in Table 5, together with the determined starting concentrations (effective solubility) for range finding tests. The final concentration of DMSO in test plate was 0.2%. For stock solutions test item Q315 was finally dissolved in 30 mM in 1.76% ammonia in water after trying to find a suitable solvent. Final concentration in test plate was 0.00352% ammonia for runs with test item Q315.

Table 5. Results from solubility testing according to SOP RISE 5569 v 2.0.

Chemical name; RISE Test item ID	Solubility in DMSO (mM)	Procedure followed to obtain solubility in DMSO	Observations on evaluated concentrations	Effective solubility (µM)
A427	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
B258	100	Vortexing 1 min	Aqueous solution, Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
C700	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
D322	N/A	Vortexing 1 min	Addressed in study plan, test item not available for solubility testing.	N/A
E073	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
F808	N/A	Vortexing 1 min	Addressed in study plan, test item not available for solubility testing.	N/A
G777	100	Vortexing 4 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
H083	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
I488	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 60 µM	60
J171	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
K047	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
L465	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
M192	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
N356	30	Sonication 15 min	Soluble in DMSO at 30 mM, soluble in buffer at 60 µM	60
O257	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200

Chemical name; RISE Test item ID	Solubility in DMSO (mM)	Procedure followed to obtain solubility in DMSO	Observations on evaluated concentrations	Effective solubility (µM)
P137	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
Q315	N/A	Sonication 15 min	Insoluble in DMSO. Soluble at 30 mM in water + 1.76% ammonia, soluble in buffer at 60 µM	60
R498	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
S074	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
T879	30	Vortexing 1 min	Soluble in DMSO at 30 mM, soluble in buffer at 60 µM	60
U778	30	Sonication 15 min	Soluble in DMSO at 30 mM, soluble in buffer at 20 µM	20
V050	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM.	200
W796	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 60 µM	60
X573	30	Sonication 15 min	Soluble in DMSO at 30 mM, soluble in buffer at 60 µM	60
AA039	100	Vortexing 1 min	soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
AB253	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 10 µM	10
AC426	100	Vortexing 1 min	soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200
AD060	100	Vortexing 1 min	Soluble in DMSO at 100 mM, soluble in buffer at 200 µM.	200
AE098	30	Sonication 15 min	Soluble in DMSO at 30 mM, soluble in buffer at 60 µM	60
AF364	100	Vortexing 1 min	soluble in DMSO at 100 mM, soluble in buffer at 200 µM	200

Concentration selection for main assay/continued range finding tests

Table 6 shows the selected concentrations and dilution factors for the main assay, in case of proven inhibitory effect, or for continued range finding tests in case no response was detected. Justification for the selection(s) is also given in each case.

Table 6. Concentration selection for main assay/continued range finding testing.

Chemical name; RISE Test item ID	[C8] selected for further testing	Dilution factor selected for further testing	Reason for concentration selection	r-C7 activity >10%
A427	200 µM	10	No inhibitory effect.	No, based on 211213:1kf
B258	200 µM	10	No effect	No, based on 211217:1kf
C700	200 µM	10	No inhibitory effect.	No, based on 220927:1kn
D322	200 µM	10	No inhibitory effect.	No, based on 211217:1kf
E073	200 µM	4	Inhibitory effect, max effect at 200 µM. 2 concentrations >70% inhibition.	No, based on 211215:1kf
F808	100 µM	3	Inhibitory effect, max effect at 200 µM. 2 concentrations >70% inhibition. Steep curve, 4 concentrations with no effect.	No, based on 211215:1kf
G777	200 µM	10	No effect	No, based on 211215:2kf
H083	120 µM	6	Inhibitory effect, max effect below 200 µM. 4 concentrations >70% inhibition.	No, based on 211217:2kf
I488	60 µM	4	Inhibitory effect, max effect at 200 µM. 1 concentrations >70% inhibition.	No, based on 211213:3kf
J171	200 µM	10	No effect	No, based on 210505:2kn
K047	200 µM	10	No effect	No, based on 210505:2kn
L465	200 µM	2	Week inhibitory effect. No concentrations >70% inhibition.	No, based on 211215:2kf
M192	200 µM	2	Week inhibitory effect. No concentrations >70% inhibition. Only one concentration >20%.	No, based on 211215:2kf
N356	60 µM	10	No effect	No, based on 211215:3kf
O257	200 µM	10	No effect	No, based on 211215:3kf
P137	200 µM	2	Week inhibitory effect. No concentrations >70% inhibition. Only one concentration >20%.	No, based on 211215:3kf
Q315	60 µM	10	No inhibitory effect.	No, based on 220822:1ap
R498	200 µM	10	No inhibitory effect.	No, based on 211216:1kf

Chemical name; RISE Test item ID	[C8] selected for further testing	Dilution factor selected for further testing	Reason for concentration selection	r-C7 activity >10%
S074	200 µM	10	No effect	No, based on 211216:1kf
T879	60 µM	10	No effect	No, based on 211216:2kf
U778	20 µM	3	Inhibitory effect. No concentrations >70% inhibition. 4 concentrations with no effect.	No, based on 211216:2kf
V050	200 µM	2	No effect. Shows week activity without test system. Test for interference.	Yes, based on 211216:2kf
W796	60 µM	5	Inhibitory effect.1 concentration <70%, 5 concentrations with no effect. Strong activity without test system. Test for interference.	Yes, based on 211216:3kf
X573	60 µM	10	No effect	No, based on 211216:3kf
AA039	200 µM	10	No effect	No, based on 211216:3kf
AB253	10 µM	10	No effect	No, based on 211217:2kf
AC426	200 µM	10	No inhibitory effect.	No, based on 211217:2kf
AD060	200 µM	5	Inhibitory effect, max effect at 200 µM. 2 concentrations >70% inhibition. 3 concentration with no effect.	No, based on 211217:3kf
AE098	60 µM	6	Inhibitory effect, max effect below 60 µM. 3 concentrations >70% inhibition. 2 concentration with no effect.	No, based on 211217:3kf
AF364	200 µM	7	Inhibitory effect. No concentrations >70% inhibition. 2 concentration with no effect.	No, based on 211217:3kf

TPO inhibition of test items

A427

Test item A427 showed no inhibitory effect in the AUR-TPO assay, see figure 15.

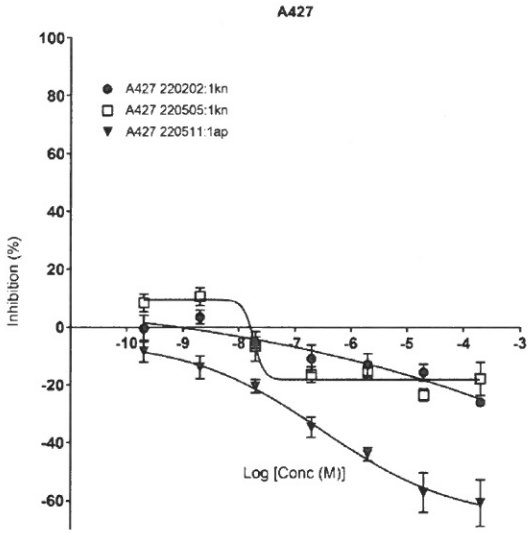


Figure 15. TPO inhibition dose-response curves for test item A427. Error bars represent the standard deviation (n=3).

B258

Test item B258 showed no inhibitory effect in the AUR-TPO assay, see figure 16.

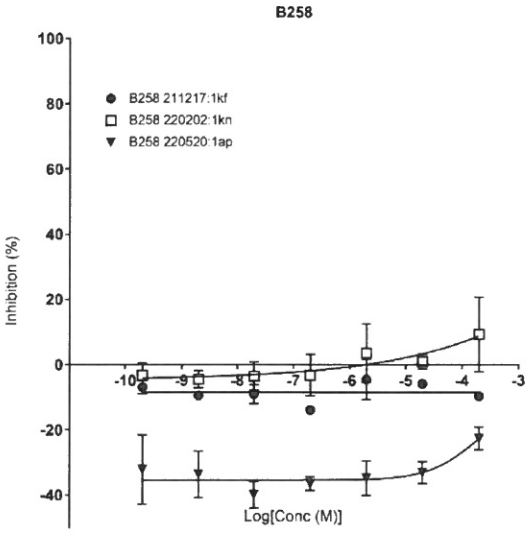


Figure 16. TPO inhibition dose-response curves for test item B258. Error bars represent the standard deviation (n=3).

C700

Test item C700 showed no inhibitory effect in the AUR-TPO assay, see figure 17.

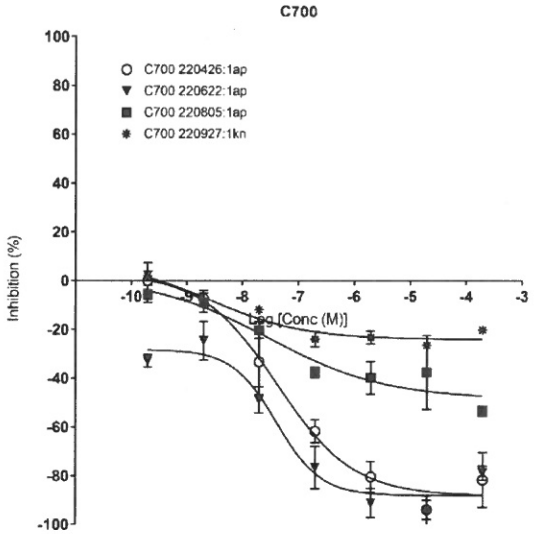


Figure 17. TPO inhibition dose-response curves for test item C700. Error bars represent the standard deviation (n=3).

D322

Test item D322 showed no inhibitory effect in the AUR-TPO assay, see figure 18.

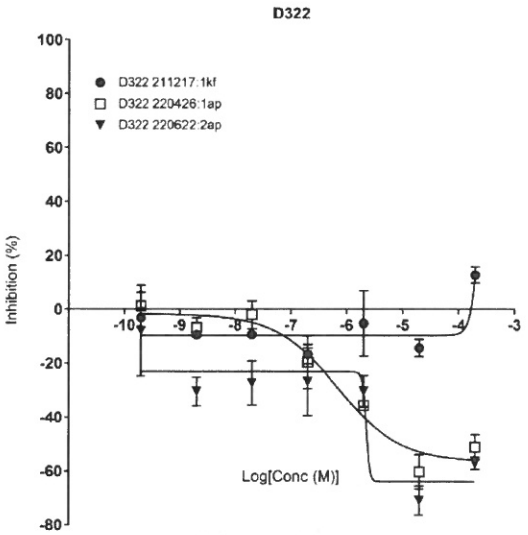


Figure 18. TPO inhibition dose-response curves for test item D322. Error bars represent the standard deviation (n=3).

E073

Test item E073 showed an inhibitory effect on TPO, see Figure 19. The inhibition was specific to TPO as the control assay QLI showed no inhibitory effect. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 7, and the parameters for the QLI runs are presented in Table 8.

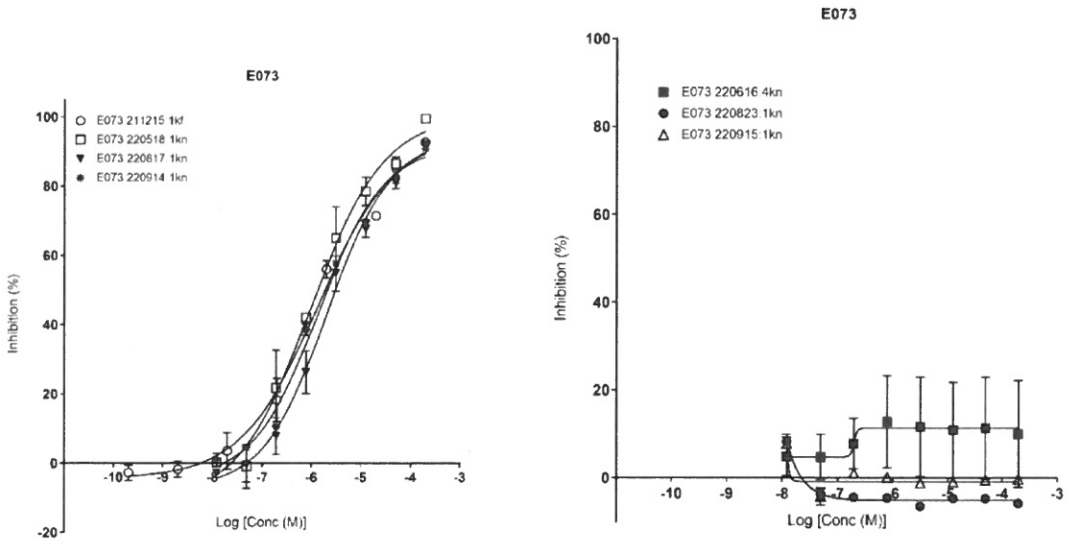


Figure 19. Left: TPO inhibition dose-response curves for test item E073. Right: QLI control assay data for test item E073. Error bars represent the standard deviation (n=3).

Table 7. Reporting of TPO inhibition data, test item E073

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 2, Plate 211215:1kf	8.3	1.36E+00	2.0%	1.15 E-01	Positive, 3 (range finding)
Run 15, Plate 220518:1kn	6.3	9.52E-01	2,1%	8.96 E-02	Positive, 6
Run 27, Plate 220817:1kn	8.4	2.05E+00	1.3%	2.65 E-01	Positive, 5
Run 34, Plate 220914:1kn	27.6	1.39E+00	1.1%	1.68 E-01	Positive, 5
Geometric mean	-	**1.39E+00**	-	**1.46E-01**	-

Table 8. Reporting of inhibition data for the QLI control assay, test item E073

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 18, Plate 220616:4kn	8595	N/A	N/A	N/A	Negative, 0
Run 29, Plate 220823:1kn	10258	N/A	N/A	N/A	Negative, 0
Run 35[1], Plate 220915:1kn	11268	N/A	N/A	N/A	Negative, 0

1. Run not valid, NC too high. The result was used anyway, since it was the last round of the control experiment and earlier data was confirmed.

F808

Test item F808 showed an inhibitory effect on TPO, see Figure 20. The inhibition was specific to TPO as the control assay QLI showed no inhibitory effect. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 9, and the parameters for the QLI runs are presented in Table 10.

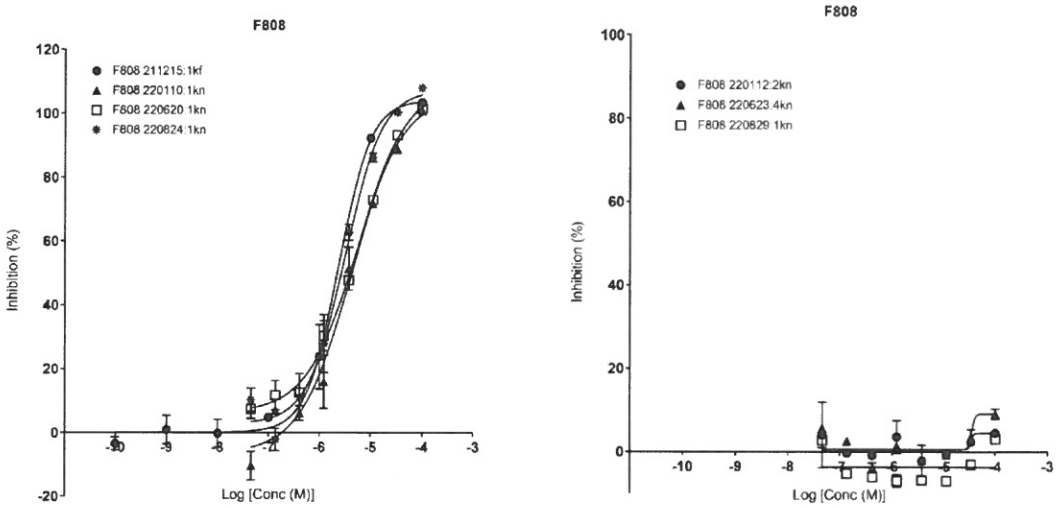


Figure 20. Left: TPO inhibition dose-response curves for test item F808. Right: QLI control assay data for test item F808. Error bars represent the standard deviation (n=3).

Table 9. Reporting of TPO inhibition data, test item F808

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 2, Plate 211215:1kf	8.3	2.37 E+00	1.0%	8.71E-01	Positive, 3 (range finding)
Run 5, Plate 220110:1kn	6.4	4.19 E+00	1.4%	8.80E-01	Positive, 4
Run 19, Plate 220620:1kn	11.8	5.52 E+00	0.83%	1.16E+00	Positive, 5
Run 30, Plate 220824:1kn	8.4	3.14 E+00	0.92%	9.93E-01	Positive, 5
Geometric mean	-	3.62E+00	-	9.69E-01	-

Table 10. Reporting of inhibition data for the QLI control assay, test item F808

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 6, Plate 220112:2kn	13216	N/A	N/A	N/A	Negative, 0
Run 23, Plate 220623:4kn	10288	N/A	N/A	N/A	Negative, 0
Run 32, Plate 220829:1kn	10451	N/A	N/A	N/A	Negative, 0

G777

Test item G777 showed no inhibitory effect in the AUR-TPO assay, see figure 21.

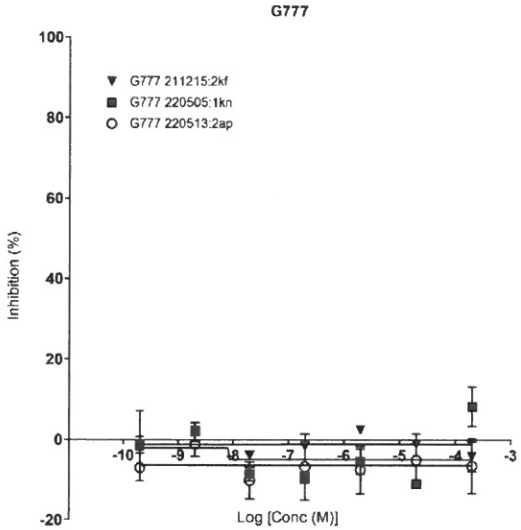


Figure 21. TPO inhibition dose-response curves for test item G777. Error bars represent the standard deviation (n=3).

H083

Test item H083 showed an inhibitory effect on TPO, see Figure 22. The inhibition was specific to TPO although the control assay QLI showed an effect at the two highest concentrations. The shift in determined IC50 values between the two assays is several orders of magnitude (see Table 33). The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 11, and the parameters for the QLI runs are presented in Table 12.

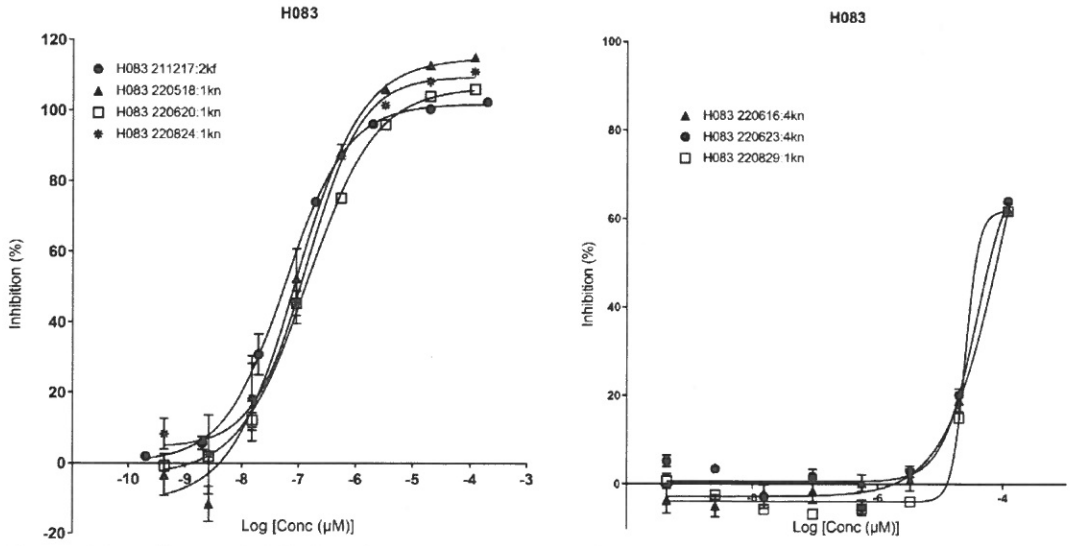


Figure 22. Left: TPO inhibition dose-response curves for test item H083. Right: QLI control assay data for test item H083. Error bars represent the standard deviation (n=3).

Table 11. Reporting of TPO inhibition data, test item H083

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 4, Plate 211217:2kf	24.0	5.86 E-02	0.45%	1.04E-02	Positive, 5 (range finding)
Run 15, Plate 220518:1kn	6.3	9.06 E-02	1.0%	1.42E-02	Positive, 5
Run 19, Plate 220620:1kn	11.8	1.44E-01	0.41%	2.13E-02	Positive, 5
Run 30, Plate 220824:1kn	8.4	1.45E-01	1.0%	3.16E-02	Positive, 5
Geometric mean	-	**9.70E-02**	-	**1.72E-02**	-

Table 12. Reporting of inhibition data for the QLI control assay, test item H083

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 18, Plate 220616:4kn	8595	1.15E+02	13%	2.50E+01	Positive, 1
Run 23, Plate 220623:4kn	10288	3.82E+01	6.2%	1.58E+01	Positive, 2
Run 32, Plate 220829:1kn	10451	~2.50E+01	Ambiguous curve fit	~1.79E+01	Positive, 1
Geometric mean[1]	-	**6.63E+01**	-	**1.99E+01**	-

1. Ambiguous curve fit values excluded

I488

Test item I488 showed an inhibitory effect on TPO, see Figure 23. The inhibition was specific to TPO as the control assay QLI showed no inhibitory effect. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 13, and the parameters for the QLI runs are presented in Table 14.

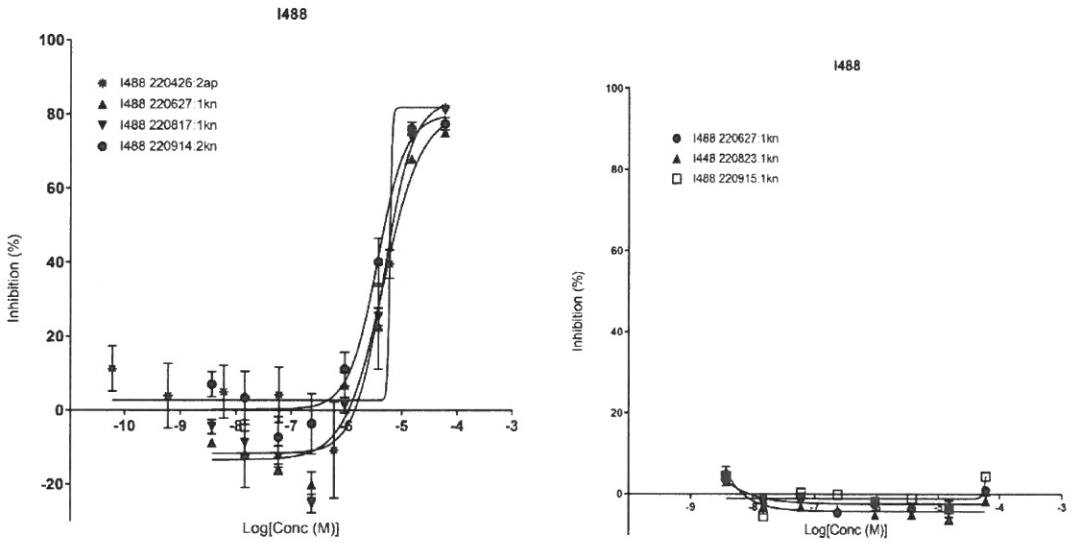


Figure 23. Left: TPO inhibition dose-response curves for test item I488. Right: QLI control assay data for test item I488. Error bars represent the standard deviation (n=3).

Table 13. Reporting of TPO inhibition data, test item I488

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 9, Plate 220426:1ap	6.6	~6.04E+00	Ambiguous curve fit	~4.91E+00	Positive, 2 (range finding)
Run 23, Plate 220627:1kn	6.7	4.56E+00	1.7%	1.51E+00	Positive, 3
Run 27, Plate 220817:1kn	8.4	4.67E+00	1.1%	2.01E+00	Positive, 3
Run 34, Plate 220914:2kn	17.1	3.57E+00	1.1%	1.57E+00	Positive, 3
Geometric mean[1]	-	**4.24E+00**	-	**1.68E+00**	-

1. Ambiguous curve fit values excluded

Table 14. Reporting of inhibition data for the QLI control assay, test item I448

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 24, Plate 220630:1kn	9750	N/A	N/A	N/A	Negative, 0
Run 29, Plate 220823:1kn	10258	N/A	N/A	N/A	Negative, 0
Run 35[1], Plate 220915:1kn	11268	N/A	N/A	N/A	Negative, 0

1. Run not valid, NC too high. The result was used anyway, since it was the last round of the control experiment and earlier data was confirmed.

J171

Test item J171 showed no inhibitory effect in the AUR-TPO assay, see figure 24.

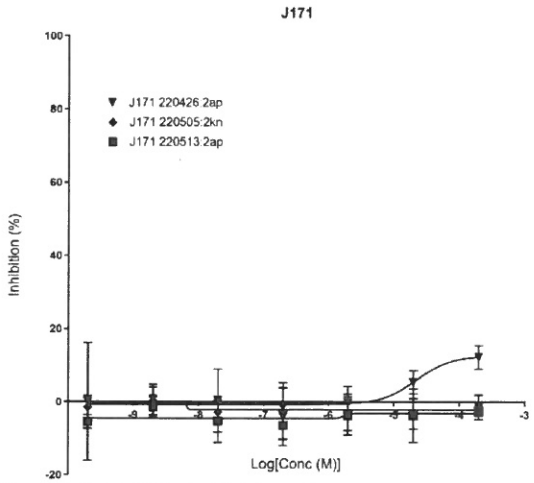


Figure 24. TPO inhibition dose-response curves for test item J171. Error bars represent the standard deviation (n=3).

K047

Test item K047 showed no inhibitory effect in the AUR-TPO assay, see figure 25.

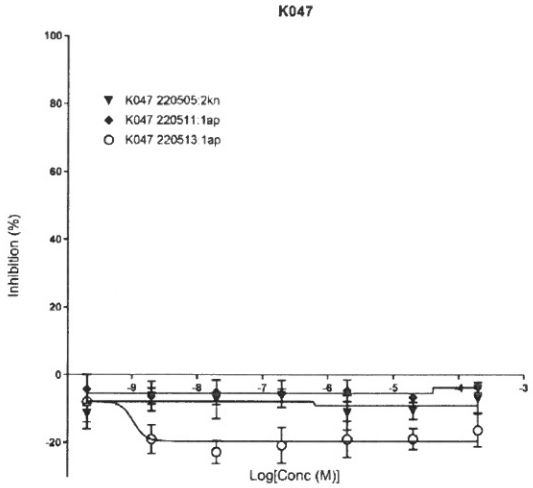


Figure 25. TPO inhibition dose-response curves for test item K047. Error bars represent the standard deviation (n=3).

L465

Test item L465 showed a weak inhibitory effect on TPO, see Figure 26. The test item also showed effect in the QLI control assay. The shift in determined IC50 values between the two assays is very small. The specificity value for the AUR-TPO assay is calculated in Table 33. The result is close to the cut-off and is thus classified as ambiguous. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 15, and the parameters for the QLI runs are presented in Table 16.

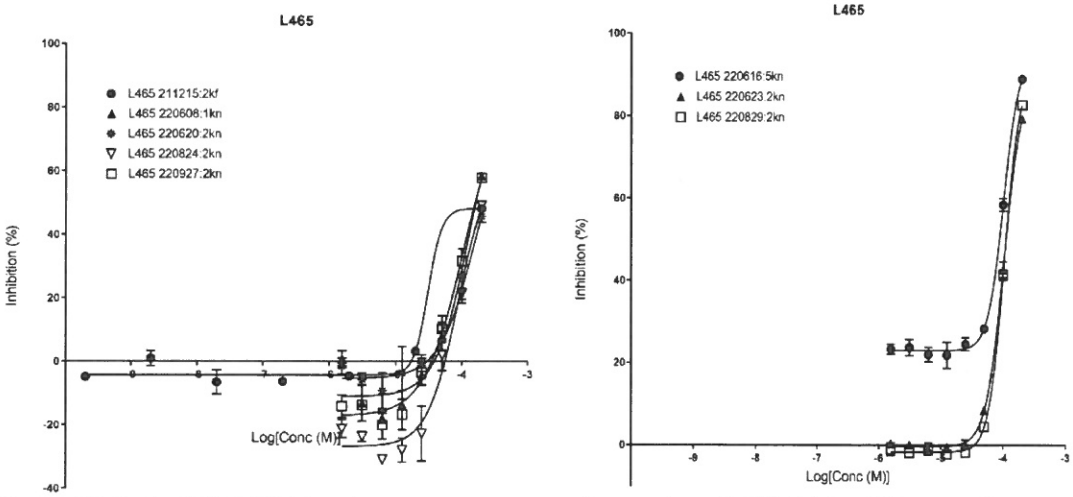


Figure 26. Left: TPO inhibition dose-response curves for test item L465. Right: QLI control assay data for test item L465. Error bars represent the standard deviation (n=3).

Table 15. Reporting of TPO inhibition data, test item L465

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 2, Plate 211215:2kf	6.4	~3.07E+01	Ambiguous curve fit	~2.19E+01	Positive, 1 (range finding)
Run 17, Plate 220608:1kn	15.2	1.26E+02	5.1%	5.68E+01	Positive, 2
Run 19, Plate 220620:2kn	8.3	1.54E+02	7.1%	6.56E+01	Positive, 2
Run 30, Plate 220824:2kn	6.9	8.58E+01	2.0%	4.16E+01	Positive, 2 (Shifted curve)
Run 36, Plate 220927:2kn	6.4	1.02E+02	3.1%	4.02E+02	Positive, 2
Geometric mean[1]	-	**1.14E+02**	-	**5.00E+01**	-

1. Ambiguous curve fit values excluded

Table 16. Reporting of inhibition data for the QLI control assay, test item L465

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 18, Plate 220616:5kn	10416	1.01E+02	0.30%	6.81E+01	Positive, 2 - if baseline start at 0
Run 22, Plate 220623:2kn	11901	1.03E+02	0.21%	6.57E+01	Positive, 2
Run 32, Plate 220829:2kn	10460	1.04E+02	0.13%	7.08E+01	Positive, 2
Geometric mean	-	**1.02E+02**	-	**6.82E+01**	-

M192

Test item M192 showed a weak inhibitory effect on TPO, see Figure 27. The test item also showed effect in the QLI control assay. The shift in determined IC50 values between the two assays is very small. The specificity value for the AUR-TPO assay is calculated in Table 33. The result is close to the cut-off and is thus classified as ambiguous. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 17, and the parameters for the QLI runs are presented in Table 18.

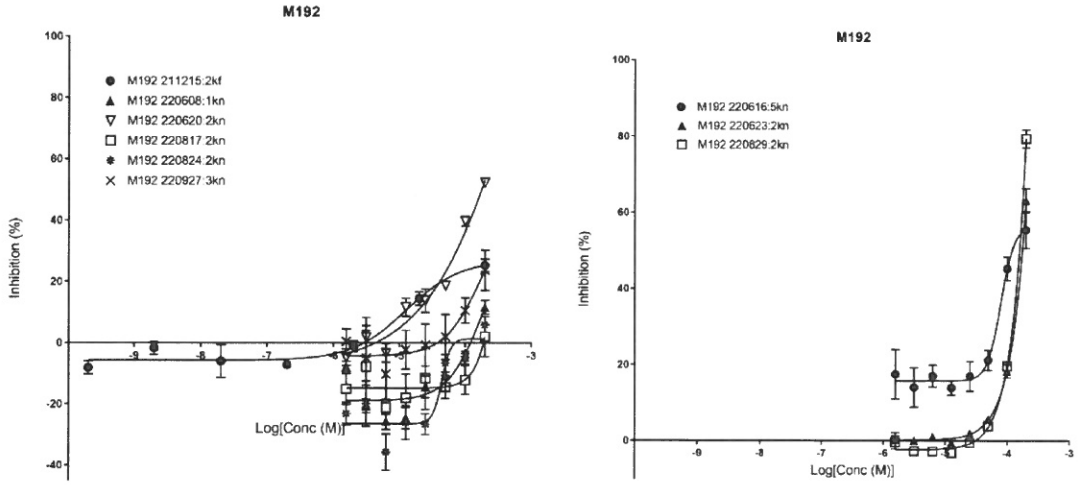


Figure 27. Left: TPO inhibition dose-response curves for test item M192. Right: QLI control assay data for test item M192. Error bars represent the standard deviation (n=3).

Table 17. Reporting of TPO inhibition data, test item M192

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 2, Plate 211215:2kf	6.4	1.25E+01	2.8%	3.37E+00	Positive, 1 (range finding)
Run 17, Plate 220608:1kn	15.2	1.87E+02	24%	7.90E+01	Negative, 0
Run 19, Plate 220620:2kn	8.3	7.65E+02	76%	8.36E+01	Positive, 2
Run 27, Plate 220817:2kn	4.6	Interrupted	Error	Interrupted	Negative, 0
Run 30, Plate 220824:2kn	6.9	4.37E+01	2.0%	3.53E+01	Negative, 0 Shifted curve
Run 36, Plate 220927:3kn	5.0	1.85E+02	28%	7.37E+01	Positive, 1
Geometric mean[1]	-	**1.08E+02**	-	**3.57E+01**	-

1. Erroneous curve fit values excluded

Table 18. Reporting of inhibition data for the QLI control assay, test item M192

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 18, Plate 220616:5kn	10416	7.88E+01	0.85%	5.60E+01	Positive, 2 (corrected for baseline shift)
Run 22, Plate 220623:2kn	11901	~3.69E+03	Ambiguous curve fit	~1.70E+03	Positive, 1
Run 32, Plate 220829:2kn	10460	~1.69E+03	Ambiguous curve fit	~8.16E+02	Positive, 1
Geometric mean[1]	-	**7.88E+01**	-	**5.60E+01**	-

1. Ambiguous curve fit values excluded

N356

Test item N356 showed no inhibitory effect in the AUR-TPO assay in two of three runs, see figure 28.

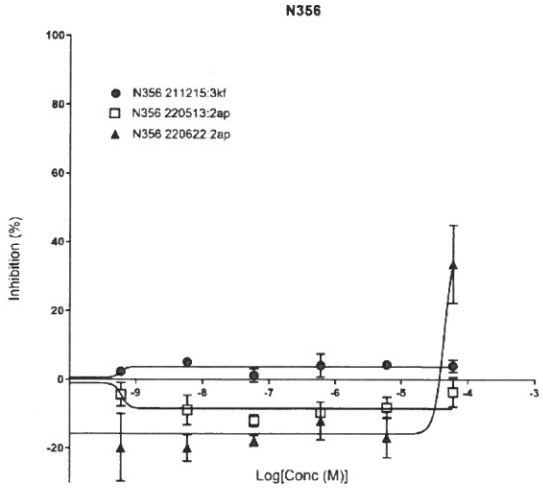


Figure 28. TPO inhibition dose-response curves for test item N356. Error bars represent the standard deviation (n=3).

O257

Test item O257 showed no inhibitory effect in the AUR-TPO assay, see figure 29.

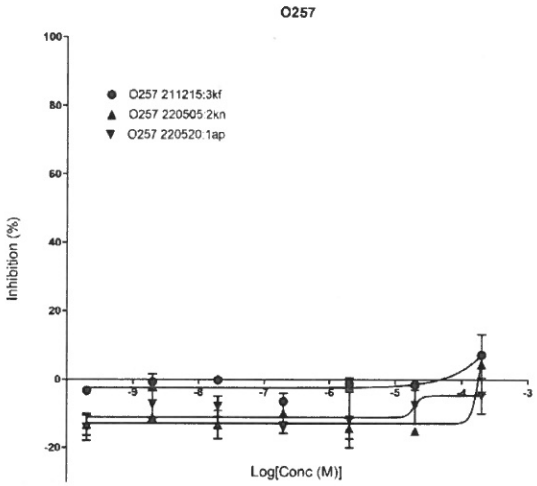


Figure 29. TPO inhibition dose-response curves for test item O257. Error bars represent the standard deviation (n=3).

P137

Test item P137 showed a weak inhibitory effect on TPO, see Figure 30. The inhibition was specific to TPO as the control assay QLI showed no inhibitory effect. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 19, and the parameters for the QLI runs are presented in Table 20.

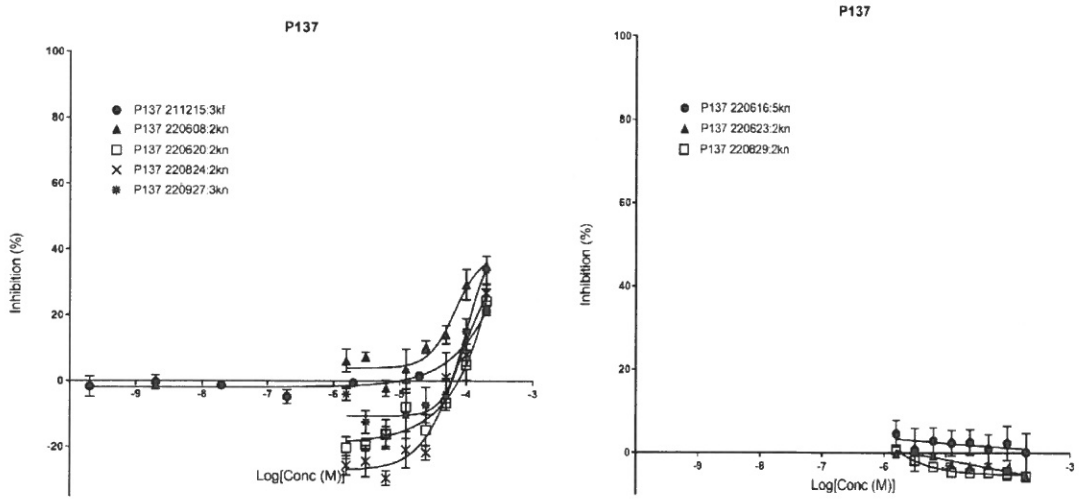


Figure 30. Left: TPO inhibition dose-response curves for test item P137. Right: QLI control assay data for test item P137. Error bars represent the standard deviation (n=3).

Table 19. Reporting of TPO inhibition data, test item P137

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 2, Plate 211215:3kf	4.6	~1.85E+03	Ambiguous curve fit	~3.87E+04	Positive, 1 (range finding)
Run 17, Plate 220608:2kn	11.5	6.67E+01	2.2%	3.58E+01	Positive, 2
Run 19, Plate 220620:2kn	8.3	~5.93E+05	Ambiguous curve fit	~1.17E+05	Positive, 1
Run 30, Plate 220824:2kn	6.9	7.71E+01	3.7%	3.07E+01	Positive, 1 Shifted curve
Run 36, Plate 220927:3kn	5.0	1.01E+02	2.5%	5.91E+01	Positive, 1
Geometric mean[1]	-	**8.06E+01**	-	**4.02E+01**	-

1. Ambiguous curve fit values excluded

Table 20. Reporting of inhibition data for the QLI control assay, test item P137

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 18, Plate 220616:5kn	10416	N/A	N/A	N/A	Negative, 0
Run 22, Plate 220623:2kn	11901	N/A	N/A	N/A	Negative, 0
Run 32, Plate 220829:2kn	10460	N/A	N/A	N/A	Negative, 0

Q315

Test item Q315 showed no inhibitory effect in the AUR-TPO assay, see figure 31.

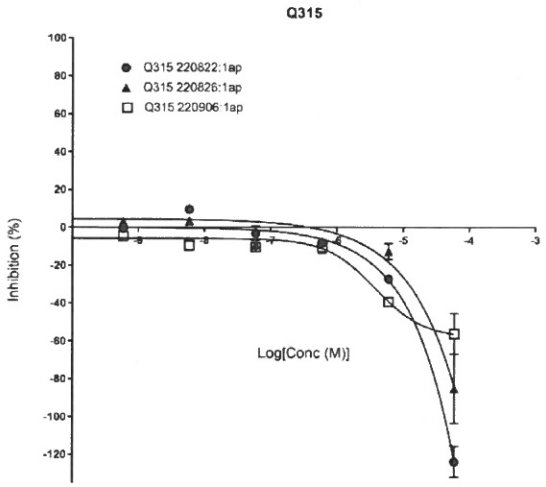


Figure 31. TPO inhibition dose-response curves for test item Q315. Error bars represent the standard deviation (n=3).

R498

Test item R498 showed no inhibitory effect in the AUR-TPO assay, see figure 32.

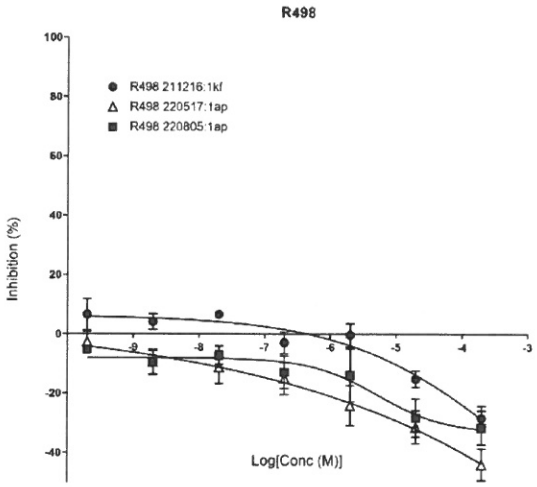


Figure 32. TPO inhibition dose-response curves for test item R498. Error bars represent the standard deviation (n=3).

S074

Test item S074 showed no inhibitory effect in the AUR-TPO assay, see figure 33.

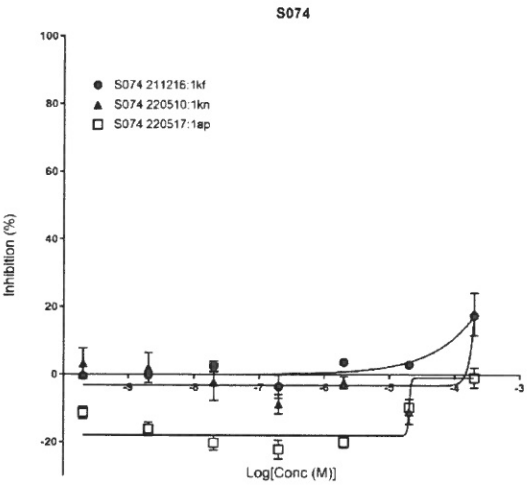


Figure 33. TPO inhibition dose-response curves for test item S074. Error bars represent the standard deviation (n=3).

T879

Test item T879 showed no inhibitory effect in the AUR-TPO assay, see figure 34.

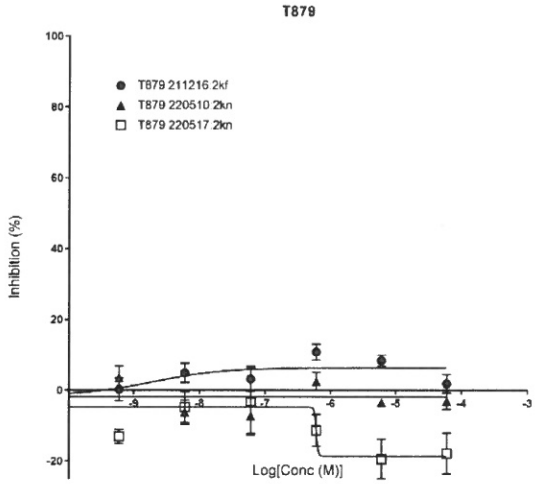


Figure 34. TPO inhibition dose-response curves for test item T879. Error bars represent the standard deviation (n=3).

U778

Test item U778 showed an inhibitory effect on TPO, see Figure 35. The test item also showed effect in the QLI control assay. The shift in determined IC50 values between the two assays is very small, indicating that the test item is a false positive for TPO inhibition. The specificity value for the AUR-TPO assay is calculated in Table 33. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 21, and the parameters for the QLI runs are presented in Table 22.

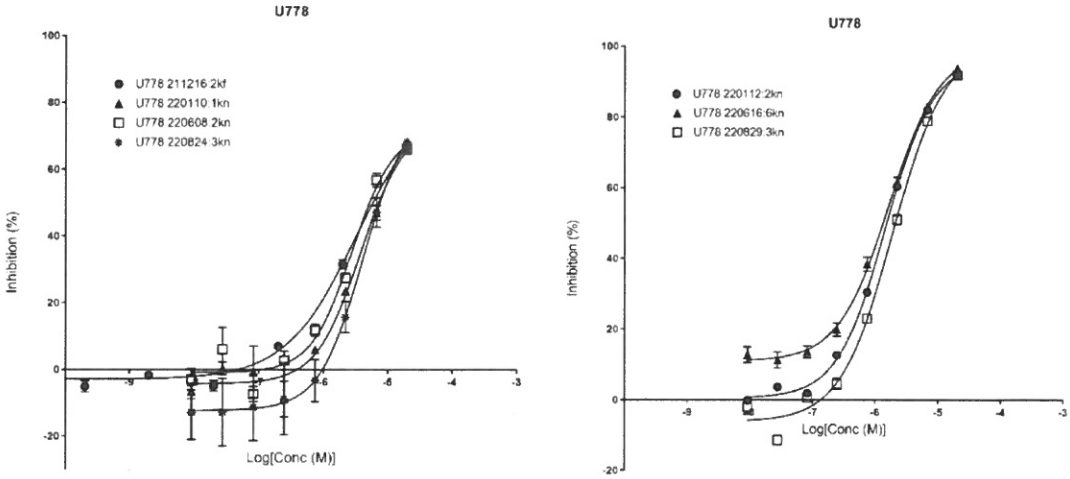


Figure 35. Left: TPO inhibition dose-response curves for test item U778. Right: QLI control assay data for test item U778. Error bars represent the standard deviation (n=3).

Table 21. Reporting of TPO inhibition data, test item U778

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 3, Plate 211216:2kf	9.6	3.33E+00	2.6%	5.73E-01	Positive, 2 (range finding)
Run 5, Plate 220110:1kn	6.4	3.76E+00	2.0%	1.25E+00	Positive, 3
Run 17, Plate 220608:2kn	11.5	2.79E+00	1.4%	1.01E+00	Positive, 3
Run 30, Plate 220824:3kn	5.1	4.17E+00	1.9%	1.42E+00	Positive, 2
Geometric mean	-	**3.47E+00**	-	**1.00E+00**	-

Table 22. Reporting of inhibition data for the QLI control assay, test item U778

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 6, Plate 220112:2kn	13216	1.44E+00	0.27%	4.31E-01	Positive, 4
Run 18, Plate 220616:6kn	8685	1.65E+00	0.44%	4.60E-01	Positive, 4
Run 32, Plate 220829:3kn	13958	1.89E+00	0.75%	5.08E-01	Positive, 4
Geometric mean	-	**1.65E+00**	-	**4.65E-01**	-

V050

Test item V050 showed no inhibitory effect in the AUR-TPO assay, see figure 36. The assay interference control showed an activity >10%, thus a main assay was performed both with and without TPO present and corrected inhibition was calculated. The inhibition only exceeded 20% for the highest concentration and only in one round. The control assay QLI showed no inhibitory effect. The parameters describing corrected inhibition data for the valid AUR-TPO runs are presented in Table 23, and the parameters for the QLI runs are presented in Table 24.

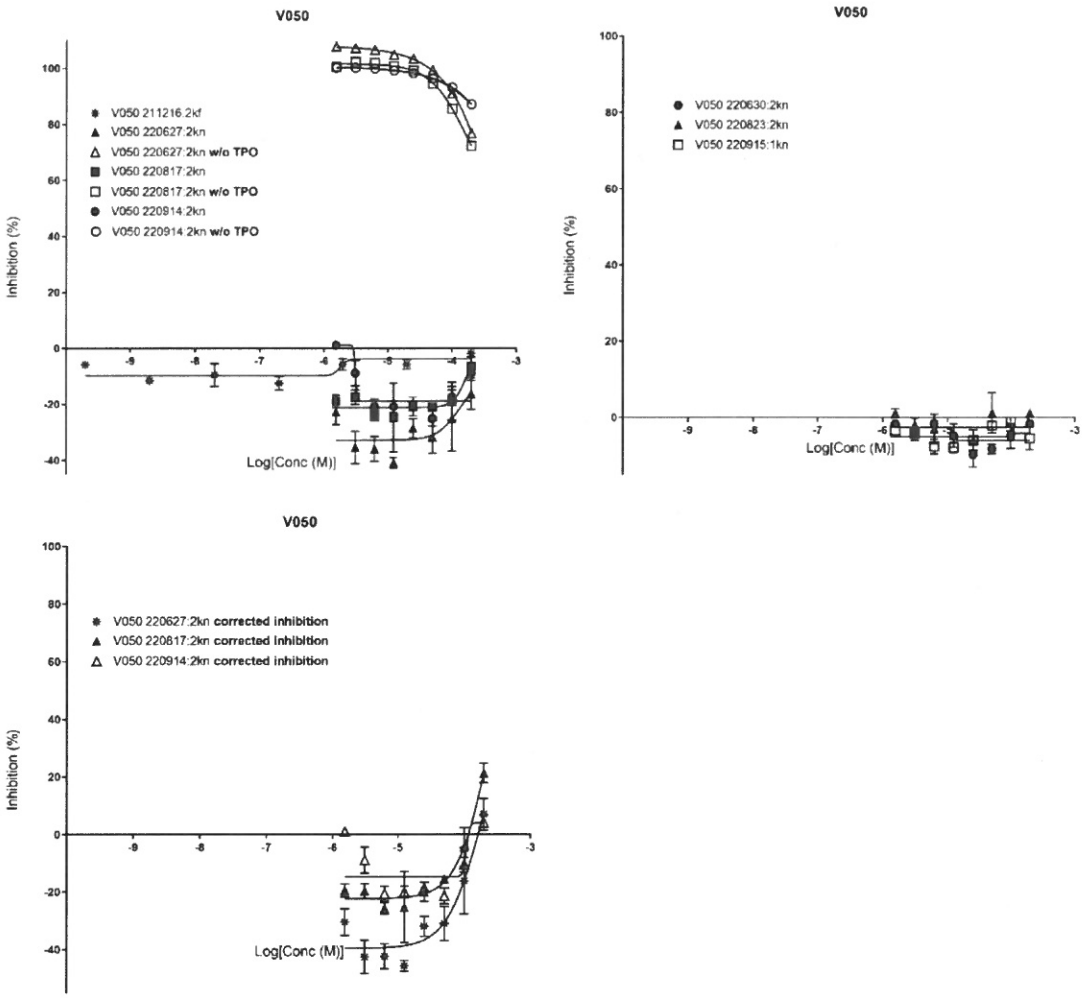


Figure 36. Top left: TPO inhibition dose-response curves for test item V050. Top right: QLI control assay data for test item V050. Bottom TPO inhibition dose-response curves after corrected inhibition were calculated for test item V050. Error bars represent the standard deviation (n=3).

Table 23. Reporting of TPO corrected inhibition data, test item V050

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 3, Plate 211216:2kf	9.6	N/A	N/A	N/A	Positive, 1 (Range finding)
Run 23, Plate 220627:2kn	4.8	1.64E+02	14%	7.37E+01	Negative, 0
Run 27, Plate 220817:2kn	4.6	2.18E+02	18%	1.03E+02	Positive, 1
Run 34, Plate 220914:2kn	17.1	~1.07E+02	Ambiguous curve fit	~9.94E+01	Negative, 0
Geometric mean[1]	-	**1.89E+02**	-	**8.69E+01**	-

1. Ambiguous curve fit values excluded

Table 24. Reporting of inhibition data for the QLI control assay, test item V050

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 24, Plate 220630:2kn	40528	N/A	N/A	N/A	Negative, 0
Run 29, Plate 220823:2kn	11545	N/A	N/A	N/A	Negative, 0
Run 35[1], Plate 220915:2kn	12272	N/A	N/A	N/A	Negative, 0

1. Run not valid, NC too high. The result was used anyway, since it was the last round of the control experiment and earlier data was confirmed.

W796

Test item W796 showed an inhibitory effect on TPO, see Figure 37. The assay interference control showed an activity >10%, thus a main assay was performed both with and without TPO present and corrected inhibition was calculated. The test item also showed an effect in the QLI control assay. The shift in determined IC50 values between the two assays is very small. The specificity value for the AUR-TPO assay is calculated in Table 33. The result is close to the cut-off value and is thus classified as ambiguous. The parameters describing corrected inhibition data for the valid AUR-TPO runs are presented in Table 25, and the parameters for the QLI runs are presented in Table 26.

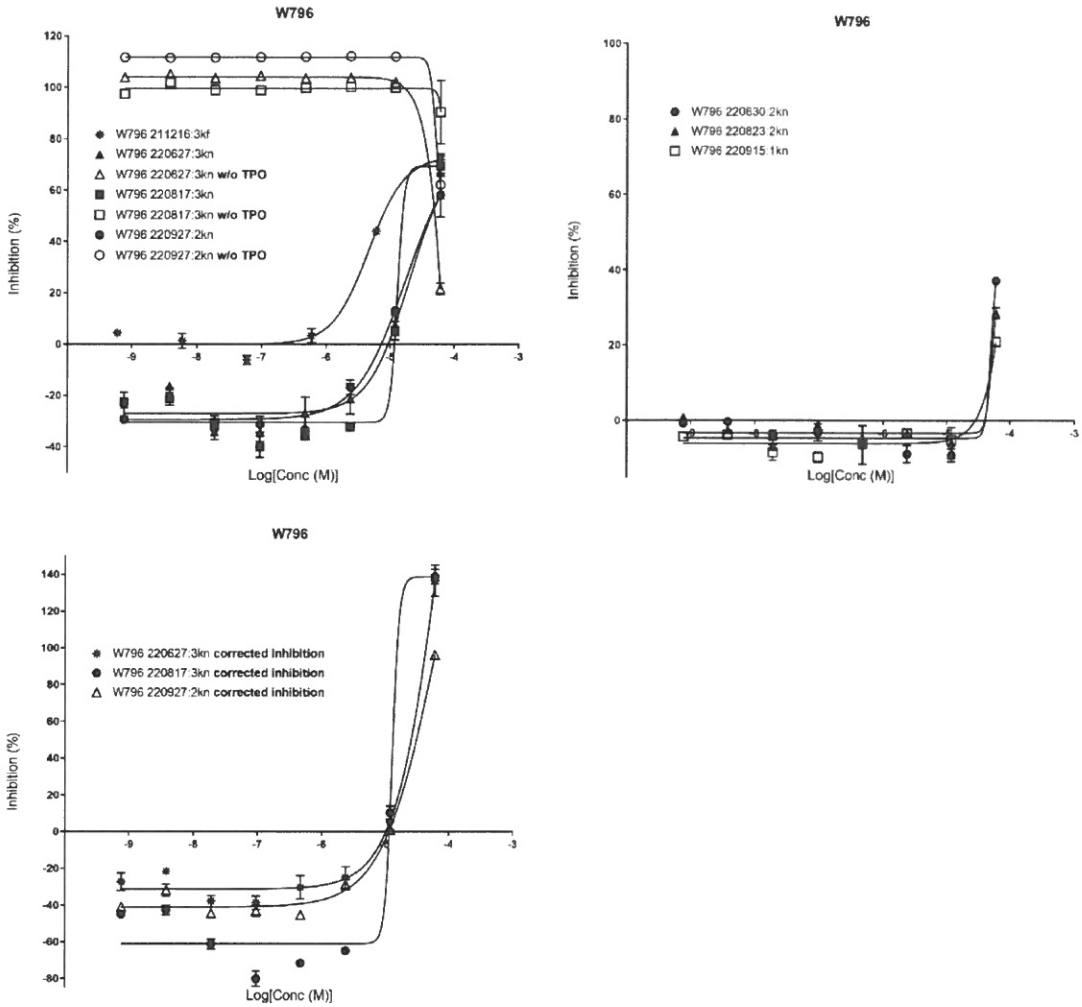


Figure 37. Top left: TPO inhibition dose-response curves for test item W796. Top right: QLI control assay data for test item W796. Bottom TPO inhibition dose-response curves after corrected inhibition were calculated for test item W796. Error bars represent the standard deviation (n=3).

Table 25. Reporting of TPO corrected inhibition data, test item W796

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 3, Plate 211216:3kf	6.9	N/A	N/A	N/A	Positive, 2 (Range finding)
Run 23, Plate 220627:3kn	3.7	8.25E+01	26%	2.62E+01	Positive, 1
Run 27, Plate 220817:3kn	3.2	~1.29E+01	Ambiguous curve fit	~1.08E+01	Positive, 1
Run 36, Plate 220927:2kn	6.4	6.47E+01	12%	1.69E+01	Positive, 1
Geometric mean[1]	-	**7.31E+01**	-	**2.10E+01**	-

1. Ambiguous curve fit values excluded

Table 26. Reporting of inhibition data for the QLI control assay, test item W796

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 24, Plate 220630:2kn	40528	~5.03E+01	Ambiguous curve fit	~1.84E+01	Positive, 1
Run 29, Plate 220823:2kn	11545	~5.11E+01	Ambiguous curve fit	~4.39E+01	Positive, 1
Run 35[1], Plate 220915:2kn	12272	~4.93E+02	Ambiguous curve fit	~2.45E+03	Positive, 1
Geometric mean[2]	-	**~1.08E+02**	-	**~5.83E+01**	-

2. Run not valid, NC too high. The result was used anyway, since it was the last round of the control experiment and earlier data was confirmed.
3. Geometric mean could not be calculated, all curve fits were ambiguous.

X573

Test item X573 showed no inhibitory effect in the AUR-TPO assay, see figure 38.

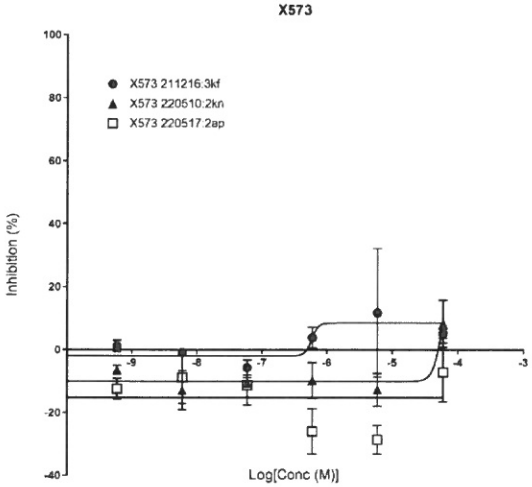


Figure 38. TPO inhibition dose-response curves for test item X573. Error bars represent the standard deviation (n=3).

AA039

Test item AA039 showed no inhibitory effect in the AUR-TPO assay, see figure 39.

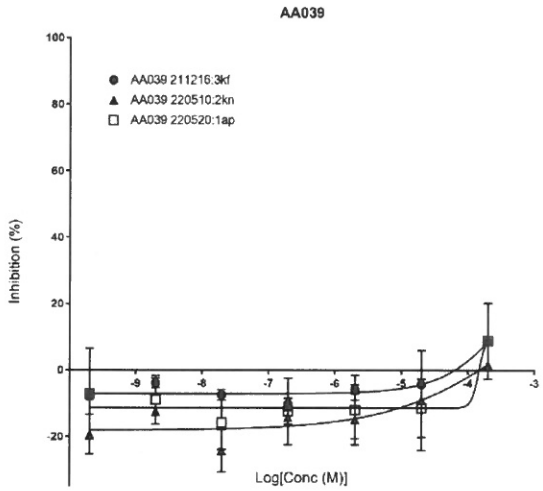


Figure 39. TPO inhibition dose-response curves for test item AA039. Error bars represent the standard deviation (n=3).

AB253

Test item AB253 showed no inhibitory effect in the AUR-TPO assay, see figure 40.

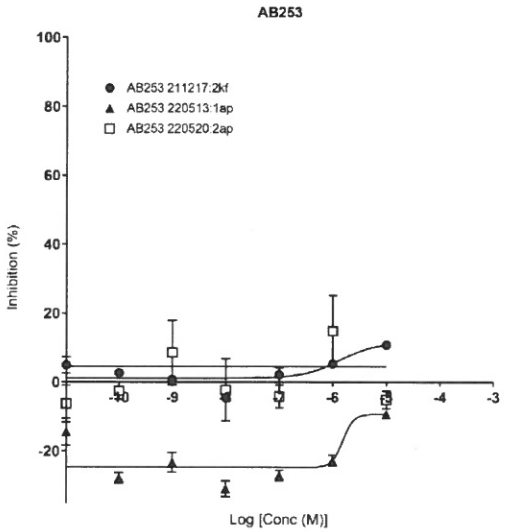


Figure 40. TPO inhibition dose-response curves for test item AB253. Error bars represent the standard deviation (n=3).

AC426

Test item AC426 showed no inhibitory effect in the AUR-TPO assay, see figure 41.

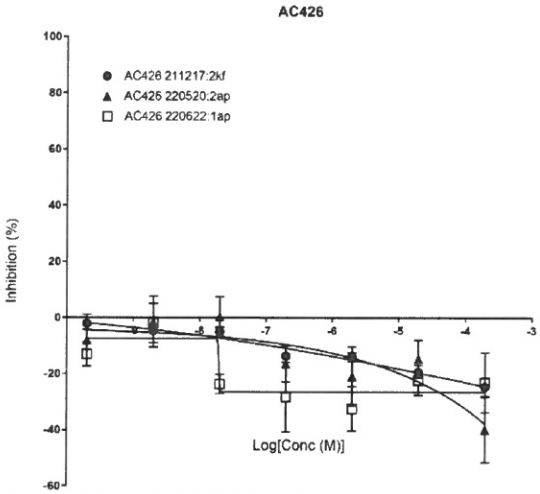


Figure 41. TPO inhibition dose-response curves for test item AC426. Error bars represent the standard deviation (n=3).

AD060

Test item AD060 showed an inhibitory effect on TPO, see Figure 42. The inhibition was specific to TPO although the control assay QLI showed an effect at the two highest concentrations. The shift in determined IC50 values between the two assays is several orders of magnitude (see Table 33). The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 27, and the parameters for the QLI runs are presented in Table 28.

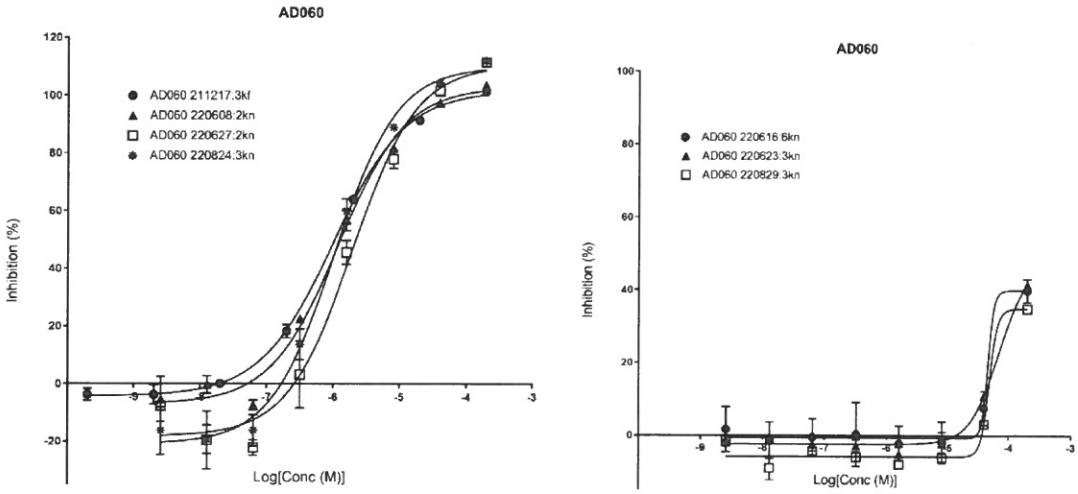


Figure 42. Left: TPO inhibition dose-response curves for test item AD060. Right: QLI control assay data for test item AD060. Error bars represent the standard deviation (n=3).

Table 27. Reporting of TPO inhibition data, test item AD060

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 4, Plate 221217:3kf	20.6	9.91E-01	0.44%	1.75E-01	Positive, 3 (range finding)
Run 17, Plate 220608:2kn	11.5	1.19E+00	0.99%	2.36E-01	Positive, 5
Run 23, Plate 220627:2kn	4.8	1.93E+00	1.3%	4.13E-01	Positive, 4
Run 30, Plate 220824:3kn	5.1	1.03E+00	0.78%	2.26E-01	Positive, 4
Geometric mean	-	**1.51E+00**	-	**2.87E-01**	-

Table 28. Reporting of inhibition data for the QLI control assay, test item AD060

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 18, Plate 220616:6kn	8685	~4.67E+01	Ambiguous curve fit	~3.99E+01	Positive, 1
Run 22, Plate 220623:3kn	12656	7.07E+01	4.34%	3.26E+01	Positive, 1
Run 32, Plate 220829:3kn	13958	~4.87E+01	Ambiguous curve fit	~3.97E+01	Positive, 1
Geometric mean[1]	-	**7.07E+01**	-	**3.26E+01**	-

2. Ambiguous curve fit values excluded

AE098

Test item AE098 showed an inhibitory effect on TPO, see Figure 43. The inhibition was specific to TPO as the control assay QLI showed no inhibitory effect. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 29, and the parameters for the QLI runs are presented in Table 30.

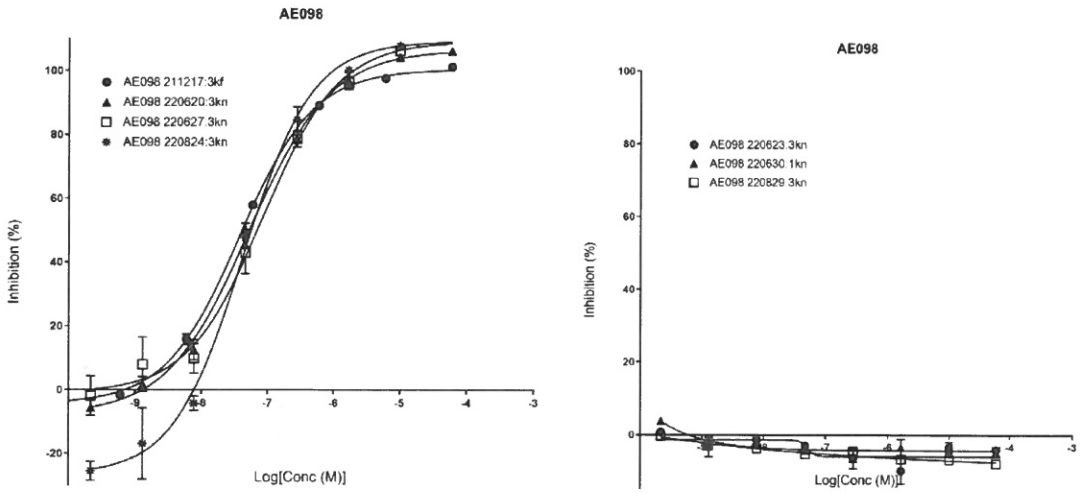


Figure 43. Left: TPO inhibition dose-response curves for test item AE098. Right: QLI control assay data for test item AE098. Error bars represent the standard deviation (n=3).

Table 29. Reporting of TPO inhibition data, test item AE098

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 4, Plate 221217:3kf	20.6	3.74E-02	0.22%	6.30E-03	Positive, 4 (range finding)
Run 19, Plate 220620:3kn	5.9	5.14E-02	0.48%	7.22E-03	Positive, 5
Run 23, Plate 220627:3kn	3.7	8.48E-02	0.93%	1.39E-02	Positive, 5
Run 30, Plate 220824:3kn	5.1	4.18E-02	0.65%	7.94E-03	Positive, 5
Geometric mean	-	**5.11E-02**	-	**8.42E-03**	-

Table 30. Reporting of inhibition data for the QLI control assay, test item AE098

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 23, Plate 220623:6kn	12656	N/A	N/A	N/A	Negative, 0
Run 24, Plate 220630:1kn	9750	N/A	N/A	N/A	Negative, 0
Run 32, Plate 220829:3kn	13958	N/A	N/A	N/A	Negative, 0

AF364

Test item AF364 showed an inhibitory effect on TPO, see Figure 44. The test item also showed effect in the QLI control assay. The shift in determined IC50 values between the two assays is very small, indicating that the test item is a false positive for TPO inhibition. The specificity value for the AUR-TPO assay is calculated in Table 33. The parameters describing inhibitory properties for the valid AUR-TPO runs are presented in Table 31, and the parameters for the QLI runs are presented in Table 32.

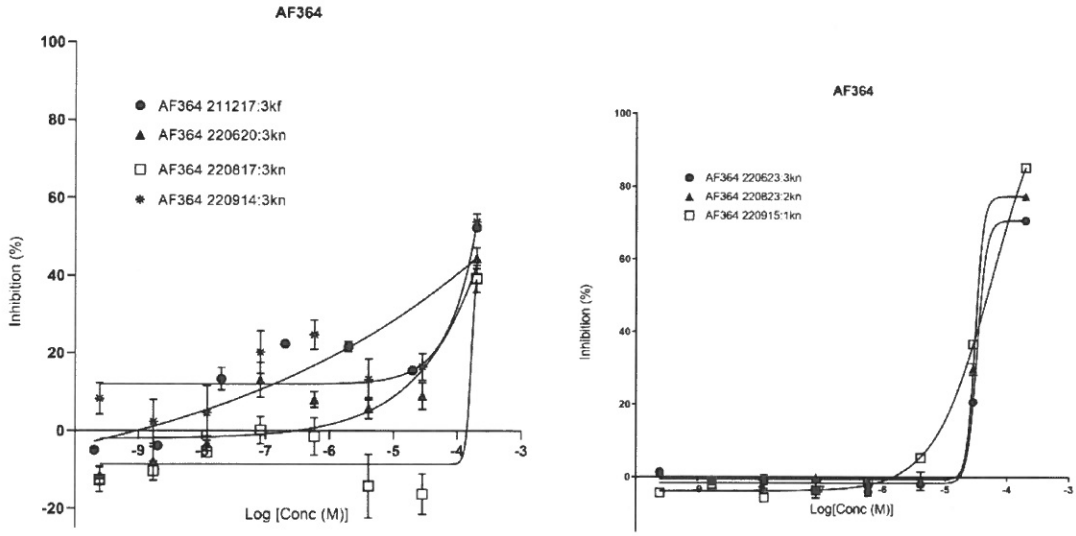


Figure 44. Left: TPO inhibition dose-response curves for test item AF364. Right: QLI control assay data for test item AF364. Error bars represent the standard deviation (n=3).

Table 31. Reporting of TPO inhibition data, test item AF364

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 4, Plate 221217:3kf	20.6	Error	Ambiguous curve fit	Error	Positive, 1 (range finding) Abberant curve (3)
Run 19, Plate 220620:3kn	5.9	Error	Ambiguous curve fit	Error	Positive, 1
Run 27, Plate 220817:3kn	3.2	~1.65E+02	Ambiguous curve fit	~1.29E+02	Positive, 1
Run 34, Plate 220914:3kn	11.8	~1.11E+02	Ambiguous curve fit	~3.01E+03	Positive, 1 Abberent curve (3)
Geometric mean[1]	-	***1.35E+03***	-	***6.23E+02***	-

1. Geometric mean calculated from two curves even though the curve fits were ambiguous.

Table 32. Reporting of inhibition data for the QLI control assay, test item AF364

Run number and plate number	Plate dynamic range	IC_{50} (µM)	CV (Log IC_{50}) (%)	IC_{20} (µM)	Classification & # concentrations > 20%
Run 23, Plate 220623:6kn	12656	~3.28E+01	Ambiguous curve fit	~2.60E+01	Positive, 2
Run 29, Plate 220823:2kn	11545	~3.06E+01	Ambiguous curve fit	~2.46E+01	Positive, 2
Run 35[1], Plate 220915:2kn	12272	5.73E+01	1.5%	1.27E+01	Positive, 2
Geometric mean[2]	-	**5.73E+01**	-	**1.27E+01**	-

1. Run not valid, NC too high. The result was used anyway, since it was the last round of the control experiment and earlier data was confirmed.
2. Ambiguous curve fit values excluded

Classification of test items

A summary of the findings is presented in Table 33.
The selectivity values used for classification is calculated according to Equation 1 and are presented in Table 33.

$$Selectivity = \min(\log(AC_{20,QLI}),3) - \log(AC_{20,AUR}) \quad (1)$$

A test item with inhibition ≥ 20% for any tested concentration and Selectivity > 0 is classified as positive in the AUR-TPO assay, and test items with inhibition ≥ 20% but Selectivity ≤ 0 is a false positive. All other results are classified as negative. However, three of the results are so close to zero that they are within margin of error and are thus classified as ambiguous.

Table 33. Determination of the selectivity value and summary of TPO inhibitory properties. Ambiguous curve fits are indicated by a "~" sign before a calculated value. Negative results are indicated by "N/A".

RISE ID, identity	AC_{20} AUR-TPO assay (µM)	AC_{20} QLI assay (µM)	Selectivity value µM	Classification
A427	N/A	Not evaluated	N/A	Negative
B258	N/A	Not evaluated	N/A	Negative
C700	N/A	Not evaluated	N/A	Negative
D322	N/A	Not evaluated	N/A	Negative
E073	1.46E-01	N/A	3.8	**Positive**
F808	9.69E-01	N/A	3.0	**Positive**
G777	N/A	Not evaluated	N/A	Negative
H083	1.72E-02	1.99E0+01[1]	3.1	**Positive**
I488	1.68E+00[1]	N/A	2.8	**Positive**
J171	N/A	Not evaluated	N/A	Negative
K047	N/A	Not evaluated	N/A	Negative
L465	5.00E+01	6.82E+01	0.1	Ambiguous
M192	3.57E+01[1]	5.60E+01[2]	0.2	Ambiguous
N356	N/A	Not evaluated	N/A	Negative
O257	N/A	Not evaluated	N/A	Negative
P137	4.02E+01[1]	N/A	1.4	**Positive**
Q315	N/A	Not evaluated	N/A	Negative
R498	N/A	Not evaluated	N/A	Negative
S074	N/A	Not evaluated	N/A	Negative
T879	N/A	Not evaluated	N/A	Negative
U778	1.00E+00	4.65E-01	-0.3	Negative, unspecific inhibitor
V050	8.69E+01[1]	N/A	1.1	**Positive**
W796	2.10E+01[1]	~5.83E+01[3]	0.4	Ambiguous
X573	N/A	Not evaluated	N/A	Negative
AA039	N/A	Not evaluated	N/A	Negative
AB253	N/A	Not evaluated	N/A	Negative
AC426	N/A	Not evaluated	N/A	Negative
AD060	2.87E-01	3.26E+01[2]	2.1	**Positive**
AE098	8.42E-03	N/A	5.1	**Positive**
AF364	~6.23E+02[3]	1.27E+01[2]	-1.7	Negative, unspecific inhibitor

1. Ambiguous curve fit for one of the runs, value excluded from geometric mean.
2. Ambiguous curve fit for all except one of the runs, no mean calculated.
3. Ambiguous curve fit for all of the runs, geometric mean calculated for non-aberrant values.

Conclusion

Valid data sets with at least three runs were obtained for all test items submitted. A summary of the results is shown in Table 33.

The proposed classifications, i.e. negative, unspecific inhibitor and positive, were insufficient to describe all test items. Three test items near the cut off value were classified as ambiguous.

The thyroperoxidase activity assay was developed for the detection of compounds with ability to inhibit the enzyme thyroperoxidas. However, six of the test items showed increasing activity with higher concentrations: A427, C700, D322, Q315, R498 and AC426. This observation is outside the scope of this report and is not further assessed. The underlying chemical and/or biological mechanisms of this effect may affect the result of the assay and further investigation is recommended.

For some runs the baseline is shifted and the lowest concentrations doesn't have an inhibition close to zero as expected. There is no acceptance criterium for this issue and the outcome might indicate that the determined number of concentrations above 20% are inaccurate.

At least three runs have been performed for each test item. Apart from the shifted curves, some of the test items have variable results between runs. It seems that test items classified as positive are more consistent whereas the ambiguous (L465, M192 and W796) as well as most of the ones that showed increasing activity (A427, C700, D322, Q315, R498 and AC426) were less consistent. Possible explanations might be: personnel became more acquainted with the assay along the time of the study, some effect of the plate layout or when running multiple plates, chemicals not compatible with the test system.

Several runs of the AUR-TPO assay and the QLI assay were invalid. Part of the explanation is that personnel were new at performing the assay. A complicated plate layout, with small volumes was manually pipetted in a limited time-range since TPO, AUR and luciferase were breaking down in room temperature. Even one small mistake would make the entire run invalid. If performed in larger scale, a pipetting robot might prevent this. In this study the solution was pipetting fewer plates in each run, which led to an increase number of runs.

Quality assurance

The quality assurance statement for this study is found in appendix 1.

The test facility has registration number 7983 and is approved according to the OECD Principles on Good Laboratory Practice (GLP) to perform *in vitro* toxicity studies with cell systems and tissues. This study was not performed under GLP. The quality of the study and of generated data was ensured by applying the following measures:

- Consistent documentation
- Internal QA review of produced data
- Change control of the applied SOPs, data analysis forms, study log templates, data recording files for the plate reader, etc.
- Validated templates for assisting work in the laboratory, e.g. study logs that perform calculations for dilution series and preparation of reagents
- Validated data analysis forms
- Calibrated and fit-for-purpose equipment and facilities
- Qualified personnel properly trained for the method according to facility routines

Records

All documents concerning this assignment will be archived at RISE for 10 years after the study completion date. After 10 years the documentation will be subject to destruction unless specific and written instruction to return them to the sponsor has been submitted to RISE.

Remaining test, reference and control items from this study will be kept until the end of the validation study (i.e. until after study 3) and if requested by the sponsor, returned to the sponsor. At the end of the validation study, all items will be disposed of unless return is requested.

References

- OECD (1998a). Organisation for Economic Co-operation and Development series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). OECD Environmental Health and Safety Publications. Environment Directorate: ENV/MC/CHEM(98)17. Paris. France: OECD.
- OECD (2018), *Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment,* No. 286, OECD Publishing, Paris, https://doi.org/10.1787/9789264304796-en.

RISE Research Institutes of Sweden AB
Methodology, Textiles and Medical Technology - Medical device evaluation

2023-01-24
Ausra Peciulyte Date
Study Personnel

2023-01-24
Sara Bogren Date
Facility Manager

2023-01-24
Karin Nydahl Date
Study Director

Appendices

1. Quality Assurance Statement
2. AUR-TPO and QLI plate layouts
3. AUR-TPO assay, data for TPO extracts, reference and control items
4. QLI assay, data for reference and control items

Appendix 1

Quality Assurance Statement

Inspections have been performed according to the quality assurance program specified in SOP RISE 5563, these are summarized below.

	Study-based Inspection(s) of 8P06603:B
Phases included:	Analysis, data, and control charts.
Date of inspection(s):	2023-01-17--18
Date of inspection report:	2023-01-18

The study report of 8P06603:B is complete and accurately reflects the conduct and raw data of the study.

RISE Research Institutes of Sweden AB
Methodology, Textiles and Medical Technology

Henrik Bäckdahl 2023-01-18

Henrik Bäckdahl
Quality Assurance

Date

Appendix 2

AUR-TPO and QLI plate layouts

Plate layouts for AUR-TPO experiments are presented in Tables A2.1-A2.2 below. Each concentration for each test item, control item and reference item were tested in at least three replicates. Vehicle control (VC): buffer spiked with solvent, DMSO for all chemicals except Q315 for which 1.76% ammonia in water was used. Blank control 1 (BC1): vehicle control, water added instead of H_2O_2. Blank control 2 (BC2): vehicle control, buffer spiked with 0.017% sodium deoxycholate instead of TPO extract. For range-finding experiments, each test item were tested at 7 different concentrations. The highest concentrations (r-C7) were also tested without test system (TPO) to detect possible test item interference with the assay reagent (e.g. TPO-independent oxidation of AUR in the presence of H_2O_2 or autofluorescence). For main assay each test item were tested at 8 different concentrations
To be able to compare multiple plates in one run, the same aliquots of vehicle control and reference item C8 respectively are used for all plates in the run.

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	MMI C1	MMI C1	MMI C1	TI1 r-C1	TI1 r-C1	TI1 r-C1	TI2 r-C1	TI2 r-C1	TI2 r-C1	VC	VC	BC1
B	MMI C2	MMI C2	MMI C2	TI1 r-C2	TI1 r-C2	TI1 r-C2	TI2 r-C2	TI2 r-C2	TI2 r-C2	VC	VC	BC1
C	MMI C3	MMI C3	MMI C3	TI1 r-C3	TI1 r-C3	TI1 r-C3	TI2 r-C3	TI2 r-C3	TI2 r-C3	VC	VC	BC1
D	MMI C4	MMI C4	MMI C4	TI1 r-C4	TI1 r-C4	TI1 r-C4	TI2 r-C4	TI2 r-C4	TI2 r-C4	VC	VC	BC1
E	MMI C5	MMI C5	MMI C5	TI1 r-C5	TI1 r-C5	TI1 r-C5	TI2 r-C5	TI2 r-C5	TI2 r-C5	NC	PC	BC1
F	MMI C6	MMI C6	MMI C6	TI1 r-C6	TI1 r-C6	TI1 r-C6	TI2 r-C6	TI2 r-C6	TI2 r-C6	NC	PC	BC1
G	MMI C7	MMI C7	MMI C7	TI1 r-C7	TI1 r-C7	TI1 r-C7	TI2 r-C7	TI2 r-C7	TI2 r-C7	NC	PC	BC1
H	MMI C8	MMI C8	MMI C8	TI1 r-C7	TI1 r-C7	TI1 r-C7	TI2 r-C7	TI2 r-C7	TI2 r-C7	BC2	BC2	BC2

Plate 2-X	1	2	3	4	5	6	7	8	9	10	11	12
A	TI3 r-C1	TI3 r-C1	TI3 r-C1	TI4 r-C1	TI4 r-C1	TI4 r-C1	TI5 r-C1	TI5 r-C1	TI5 r-C1	VC	MMI C8	BC1
B	TI3 r-C2	TI3 r-C2	TI3 r-C2	TI4 r-C2	TI4 r-C2	TI4 r-C2	TI5 r-C2	TI5 r-C2	TI5 r-C2	VC	MMI C8	BC1
C	TI3 r-C3	TI3 r-C3	TI3 r-C3	TI4 r-C3	TI4 r-C3	TI4 r-C3	TI5 r-C3	TI5 r-C3	TI5 r-C3	VC	MMI C8	BC1
D	TI3 r-C4	TI3 r-C4	TI3 r-C4	TI4 r-C4	TI4 r-C4	TI4 r-C4	TI5 r-C4	TI5 r-C4	TI5 r-C4	VC	MMI C8	BC1
E	TI3 r-C5	TI3 r-C5	TI3 r-C5	TI4 r-C5	TI4 r-C5	TI4 r-C5	TI5 r-C5	TI5 r-C5	TI5 r-C5	VC	MMI C8	BC1
F	TI3 r-C6	TI3 r-C6	TI3 r-C6	TI4 r-C6	TI4 r-C6	TI4 r-C6	TI5 r-C6	TI5 r-C6	TI5 r-C6	VC	MMI C8	BC1
G	TI3 r-C7	TI3 r-C7	TI3 r-C7	TI4 r-C7	TI4 r-C7	TI4 r-C7	TI5 r-C7	TI5 r-C7	TI5 r-C7	VC	MMI C8	BC1
H	TI3 r-C7	TI3 r-C7	TI3 r-C7	TI4 r-C7	TI4 r-C7	TI4 r-C7	TI5 r-C7	TI5 r-C7	TI5 r-C7	BC2	BC2	BC2

Figure A2.1 Plate layout for the AUR-TPO assay, range finding experiments. VC: Vehicle control, NC: negative control BC3, PC: positive control PTU, BC1: H_2O_2 free wells, BC2: TPO-free wells, TIx r-Cy: test item x at 7 different range-finding concentrations, MMI C#: reference item at different concentrations. Grey cells contain the test system (TPO), white cells are without test system.

Appendix 2

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	MMI C1	MMI C1	MMI C1	TI1 C1	TI1 C1	TI1 C1	TI2 C1	TI2 C1	TI2 C1	VC	VC	BC1
B	MMI C2	MMI C2	MMI C2	TI1 C2	TI1 C2	TI1 C2	TI2 C2	TI2 C2	TI2 C2	VC	VC	BC1
C	MMI C3	MMI C3	MMI C3	TI1 C3	TI1 C3	TI1 C3	TI2 C3	TI2 C3	TI2 C3	VC	VC	BC1
D	MMI C4	MMI C4	MMI C4	TI1 C4	TI1 C4	TI1 C4	TI2 C4	TI2 C4	TI2 C4	VC	VC	BC1
E	MMI C5	MMI C5	MMI C5	TI1 C5	TI1 C5	TI1 C5	TI2 C5	TI2 C5	TI2 C5	NC	PC	BC1
F	MMI C6	MMI C6	MMI C6	TI1 C6	TI1 C6	TI1 C6	TI2 C6	TI2 C6	TI2 C6	NC	PC	BC1
G	MMI C7	MMI C7	MMI C7	TI1 C7	TI1 C7	TI1 C7	TI2 C7	TI2 C7	TI2 C7	NC	PC	BC1
H	MMI C8	MMI C8	MMI C8	TI1 C8	TI1 C8	TI1 C8	TI2 C8	TI2 C8	TI2 C8	BC2	BC2	BC2

Plate 2-X	1	2	3	4	5	6	7	8	9	10	11	12
A	TI3 C1	TI3 C1	TI3 C1	TI4 C1	TI4 C1	TI4 C1	TI5 C1	TI5 C1	TI5 C1	VC	MMI C8	BC1
B	TI3 C2	TI3 C2	TI3 C2	TI4 C2	TI4 C2	TI4 C2	TI5 C2	TI5 C2	TI5 C2	VC	MMI C8	BC1
C	TI3 C3	TI3 C3	TI3 C3	TI4 C3	TI4 C3	TI4 C3	TI5 C3	TI5 C3	TI5 C3	VC	MMI C8	BC1
D	TI3 C4	TI3 C4	TI3 C4	TI4 C4	TI4 C4	TI4 C4	TI5 C4	TI5 C4	TI5 C4	VC	MMI C8	BC1
E	TI3 C5	TI3 C5	TI3 C5	TI4 C5	TI4 C5	TI4 C5	TI5 C5	TI5 C5	TI5 C5	VC	MMI C8	BC1
F	TI3 C6	TI3 C6	TI3 C6	TI4 C6	TI4 C6	TI4 C6	TI5 C6	TI5 C6	TI5 C6	VC	MMI C8	BC1
G	TI3 C7	TI3 C7	TI3 C7	TI4 C7	TI4 C7	TI4 C7	TI5 C7	TI5 C7	TI5 C7	VC	MMI C8	BC1
H	TI3 C8	TI3 C8	TI3 C8	TI4 C8	TI4 C8	TI4 C8	TI5 C8	TI5 C8	TI5 C8	BC2	BC2	BC2

Figure A2.2. Plate layout for the AUR-TPO assay, main experiment. If more than 5 test items are tested, plate 3 are prepared with the same layout as plate 2. VC: Vehicle control, NC: negative control, PC: positive control, BC1: H_2O_2 free wells, BC2: TPO-free wells, TIx Cy: test item x at 8 different concentrations selected to cover the AUR-TPO assay response, MMI Cy: reference item at 8 different concentrations.

Plate layouts used in QLI experiments are presented in Table A2-3 below. Each concentration for each test item, control item and reference item were tested in at least three replicates. Vehicle control (VC): buffer spiked with solvent, DMSO. Blank control (BC): vehicle control, buffer added instead of test system (recombinant Quantilum luciferase). Each test item were tested at the same 8 concentrations as in AUR-TPO main assay. The highest concentrations (C8) were also tested with only buffer instead of test system, to detect possible test item interference with the assay reagent.
To be able to compare multiple plates in one run, the same aliquots of vehicle control and reference item C8 respectively are used for all plates in the run.

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	LUCINH2 C1	LUCINH2 C1	LUCINH2 C1	TI1 C1	TI1 C1	TI1 C1	TI2 C1	TI2 C1	TI2 C1	VC	VC	VC
B	LUCINH2 C2	LUCINH2 C2	LUCINH2 C2	TI1 C2	TI1 C2	TI1 C2	TI2 C2	TI2 C2	TI2 C2	VC	VC	VC
C	LUCINH2 C3	LUCINH2 C3	LUCINH2 C3	TI1 C3	TI1 C3	TI1 C3	TI2 C3	TI2 C3	TI2 C3	NC	NC	NC
D	LUCINH2 C4	LUCINH2 C4	LUCINH2 C4	TI1 C4	TI1 C4	TI1 C4	TI2 C4	TI2 C4	TI2 C4	PC	PC	PC
E	LUCINH2 C5	LUCINH2 C5	LUCINH2 C5	TI1 C5	TI1 C5	TI1 C5	TI2 C5	TI2 C5	TI2 C5	BC	BC	BC
F	LUCINH2 C6	LUCINH2 C6	LUCINH2 C6	TI1 C6	TI1 C6	TI1 C6	TI2 C6	TI2 C6	TI2 C6	BC	BC	BC
G	LUCINH2 C7	LUCINH2 C7	LUCINH2 C7	TI1 C7	TI1 C7	TI1 C7	TI2 C7	TI2 C7	TI2 C7	TI1 C8	TI1 C8	TI1 C8
H	LUCINH2 C8	LUCINH2 C8	LUCINH2 C8	TI1 C8	TI1 C8	TI1 C8	TI2 C8	TI2 C8	TI2 C8	TI2 C8	TI2 C8	TI2 C8

Plate 2-X	1	2	3	4	5	6	7	8	9	10	11	12
A	TI3 C1	TI3 C1	TI3 C1	TI4 C1	TI4 C1	TI4 C1	TI5 C1	TI5 C1	TI5 C1	VC	VC	VC
B	TI3 C2	TI3 C2	TI3 C2	TI4 C2	TI4 C2	TI4 C2	TI5 C2	TI5 C2	TI5 C2	VC	VC	VC
C	TI3 C3	TI3 C3	TI3 C3	TI4 C3	TI4 C3	TI4 C3	TI5 C3	TI5 C3	TI5 C3	LUCINH2 C8	LUCINH2 C8	LUCINH2 C8
D	TI3 C4	TI3 C4	TI3 C4	TI4 C4	TI4 C4	TI4 C4	TI5 C4	TI5 C4	TI5 C4	BC	BC	BC
E	TI3 C5	TI3 C5	TI3 C5	TI4 C5	TI4 C5	TI4 C5	TI5 C5	TI5 C5	TI5 C5	BC	BC	BC
F	TI3 C6	TI3 C6	TI3 C6	TI4 C6	TI4 C6	TI4 C6	TI5 C6	TI5 C6	TI5 C6	TI3 C8	TI3 C8	TI3 C8
G	TI3 C7	TI3 C7	TI3 C7	TI4 C7	TI4 C7	TI4 C7	TI5 C7	TI5 C7	TI5 C7	TI4 C8	TI4 C8	TI4 C8
H	TI3 C8	TI3 C8	TI3 C8	TI4 C8	TI4 C8	TI4 C8	TI5 C8	TI5 C8	TI5 C8	TI5 C8	TI5 C8	TI5 C8

Figure A2.3. Plate layout for the QLI. If more than 5 test items are tested, plate 3 and subsequent plates are prepared with the same layout as plate 2. VC: Vehicle control, NC: negative control, PC: positive control, BC: blank, i.e. test system-free wells, TIx Cy: test item x at 8 different concentrations, LUCINH2 Cx: Reference item at 8 different concentrations. Grey wells contain the test system (luciferase), white cells are without the test system (contain buffer instead).

Appendix 3

AUR-TPO assay, data for reference and control items

Data from quality control of TPO extract and which batch of TPO extract used for each experiment, are presented in Table A3.1.

Table A3.1. Batch of TPO extract used for each experiment and quality control data for each batch of TPO extract.

Exp #	Plate ID	Batch TPO extract	Determined protein concentration (µg/µl)	QC performed	MMI AC_{50} (µM) at QC	TPO efficiency at QC
1	211213:1-3kf	TPO FTC-238/hrTPO 200518:1/ TPO FTC-238/hrTPO 200518:2	1.6 / 1.0	201113/ 210412	3.2E-02/ 2.9E-02	60.3/ 20.5
2	211215:1-3kf	TPO FTC-238/hrTPO 200518:1	1.0	201113	3.2E-02	60.3
3	211216:1-3kf	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
4	211217:1-3kf	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
5	220110:1kn	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
6	220202:1-3kn	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
7	220210:1-3ap	TPO FTC-238/hrTPO 200914	2.2	210629	1.4E-01	17.8
8	220426:1-2ap	TPO FTC-238/hrTPO 200914	2.2	210629	1.4E-01	17.8
9	220505:1-2kn	TPO FTC-238/hrTPO 200914	2.2	210629	1.4E-01	17.8
10	220510:1-2kn	TPO FTC-238/hrTPO 200914	2.2	210629	1.4E-01	17.8
11	220511:1-2ap	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
12	220513:1-2ap	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
13	220517:1-2ap	TPO FTC-238/hrTPO 200907	1.5	210512	2.2E-01	20.6
14	220518:1-2kn	TPO FTC-238/hrTPO 200907	1.5	210512	2.2E-01	20.6
15	220520:1-2ap	TPO FTC-238/hrTPO 200910	1.9	210630	7.6E-02	17.1
16	220608:1-2kn	TPO FTC-238/hrTPO 220512	1.3	220608	6.0E-02	27.9
17	220620:1-3kn	TPO FTC-238/hrTPO 220516	1.1	220620	1.3E-01	18.6
18	220622:1-2ap	TPO FTC-238/hrTPO 220519	1.2	220622	7.0E-02	9.7
19	220627:1kn	TPO FTC-238/hrTPO 220523	1.2	220627	1.1E-01	14.6
20	220805:1ap	TPO FTC-238/hrTPO 220519	1.2	220622	7.0E-02	9.7
21	220816:1ap	TPO FTC-238/hrTPO 220519	1.2	220622	7.0E-02	9.7
22	220817:1-3kn	TPO FTC-238/hrTPO 220516	1.1	220620	1.3E-01	18.6
23	220822:1ap	TPO FTC-238/hrTPO 220519	1.2	220622	7.0E-02	9.7
24	220824:1-3kn	TPO FTC-238/hrTPO 220523	1.2	220627	1.1E-01	14.6
25	220826:1ap	TPO FTC-238/hrTPO 220519	1.2	220622	7.0E-02	9.7
26	220906:1ap	TPO FTC-238/hrTPO 220519	1.2	220622	7.0E-02	9.7
27	220914:1-3kn	TPO FTC-238/hrTPO 220512	1.3	220608	6.0E-02	27.9
28	220927:1-3kn	TPO FTC-238/hrTPO 220516	1.1	220620	1.3E-01	18.6

Data for acceptance criteria, reference item MMI, negative control item BP3 and positive control item PTU are presented in tables A3.2-A3.4 below. The data corresponds to Figures 3-9 in the report.

Appendix 3

Table A3.2. Data for acceptance criteria, corresponding to Figures 3-7 in the report.

Exp #	Plate ID	TPO efficiency	Plate dynamic range	Z-factor	AC_{50} (µM)	CV (VC) %	Experiment accepted?	Plate accepted?
1	211213:1kf	37.9	12.3	0.9	4.8E-02	1.8	No[1]	No[1]
1	211213:2kf	33.5	12.2	0.9	N/A	1.7	-	No[1]
1	211213:3kf	21.4	9.5	0.8	N/A	4.9	-	No[1]
2	211215:1kf	21.2	8.3	0.9	1.6E-01	2.7	Yes	Yes
2	211215:2kf	9.9	6.4	0.9	N/A	2.1	-	Yes
2	211215:3kf	6.5	4.6	0.9	N/A	1.9	-	Yes
3	211216:1kf	33.4	11.4	0.9	8.5E-02	2.6	Yes	Yes
3	211216:2kf	18.5	9.6	0.9	N/A	3.8	-	Yes
3	211216:3kf	10.9	6.9	0.9	N/A	2.7	-	Yes
4	211217:1kf	53.6	22.2	0.9	6.5E-02	3.4	Yes	Yes
4	211217:2kf	54.3	24.0	0.9	N/A	2.2	-	Yes
4	211217:3kf	41.9	20.6	0.9	N/A	2.4	-	Yes
5	220110:1kn	6.9	6.4	0.9	1.5E-01	3.0	Yes	Yes
6	220202:1kn	3.5	6.7	0.8	1.2E-01	5.9	Yes	Yes
6	220202:2kn	2.7	3.2	0.7	N/A	4.5	-	No[2]
6	220202:3kn	2.2	2.6	0.8	N/A	2.7	-	No[2]
7	220210:1ap	2.4	2.5	0.7	1.2E-01	5.2	No[2]	No[2]
7	220210:2ap	2.0	2.1	0.7	N/A	3.7	-	No[2]
7	220210:3ap	2.0	1.9	0.6	N/A	5.0	-	No[2]
8	220426:1ap	3.3	6.6	0.7	4.3E-01	6.3	Yes	Yes
8	220426:2ap	3.2	6.4	0.7	N/A	5.6	-	Yes
9	220505:1kn	13.0	12.9	0.7	1.4E-01	7.7	Yes	Yes
9	220505:2kn	5.3	5.5	0.8	N/A	4.8	-	Yes
10	220510:1kn	10.6	9.9	0.8	6.7E-02	4.3	Yes	Yes
10	220510:2kn	6.4	5.9	0.7	N/A	6.7	-	Yes
11	220511:1ap	7.7	8.3	0.9	1.5E-01	2.1	Yes	Yes
11	220511:2ap	6.0	1.8	0.8	N/A	4.3	-	No[3]
12	220513:1ap	5.9	7.7	0.8	1.5E-01	5.1	Yes	Yes
12	220513:2ap	4.6	6.8	0.9	N/A	2.8	-	Yes
13	220517:1ap	10.4	17.6	0.9	9.8E-02	3.8	Yes	Yes
13	220517:2ap	12.4	17.5	0.7	N/A	7.6	-	Yes
14	220518:1kn	9.6	6.3	0.6	5.9E-02	5.0	Yes	Yes
14	220518:2kn	2.9	3.7	0.8	N/A	2.2	-	No[2]
15	220520:1ap	7.7	7.4	0.9	1.7E-01	1.8	Yes	Yes
15	220520:2ap	7.4	6.8	0.8	N/A	5.8	-	Yes
16	220608:1kn	27.9	15.2	0.9	6.0E-02	4.6	Yes	Yes
16	220608:2kn	18.2	11.5	0.8	N/A	4.7	-	Yes
17	220620:1kn	18.6	11.8	0.9	1.3E-01	4.4	Yes	Yes
17	220620:2kn	12.3	8.3	0.9	N/A	3.0	-	Yes
17	220620:3kn	7.7	5.9	0.8	N/A	3.3	-	Yes
18	220622:1ap	9.7	9.4	0.7	7.0E-02	9.2	Yes	Yes
18	220622:2ap	11.0	10.0	0.8	N/A	6.3	-	Yes
19	220627:1kn	14.6	6.7	0.8	1.1E-01	5.8	Yes	Yes
19	220627:2kn	6.8	4.8	0.8	N/A	5.6	-	Yes
19	220627:3kn	4.4	3.7	0.7	N/A	9.0	-	Yes

Appendix 3

Exp #	Plate ID	TPO efficiency	Plate dynamic range	Z-factor	AC_{50} (µM)	CV (VC) %	Experiment accepted?	Plate accepted?
20	220805:1ap	13.9	7.3	0.8	5.0E-02	5.6	Yes	Yes
21	220816:1ap	15.0	7.6	0.8	1.2E-01	4.3	No[4]	Yes
22	220817:1kn	12.0	8.4	0.9	6.7E-02	2.2	Yes	Yes
22	220817:2kn	5.2	4.6	0.9	N/A	3.2	-	Yes
22	220817:3kn	3.4	3.2	0.7	N/A	6.3	-	Yes
23	220822:1ap	12.3	5.8	0.9	4.5E-02	4.1	Yes	Yes
24	220824:1kn	27.7	8.4	0.8	2.6E-02	5.7	Yes	Yes
24	220824:2kn	14.7	6.9	0.8	N/A	5.4	-	Yes
24	220824:3kn	8.0	5.1	0.9	N/A	2.8	-	Yes
25	220826:1ap	20.3	6.8	0.9	3.6E-02	2.9	Yes	Yes
26	220906:1ap	20.4	12.5	0.8	2.4E-02	4.8	Yes	Yes
27	220914:1kn	27.9	27.6	0.9	4.9E-02	5.9	Yes	Yes
27	220914:2kn	18.0	17.1	0.8	N/A	3.9	-	Yes
27	220914:3kn	12.3	11.8	0.8	N/A	7.6	-	Yes
28	220927:1kn	25.6	9.1	0.9	4.0E-02	4.5	Yes	Yes
28	220927:2kn	16.0	6.4	0.7	N/A	3.7	-	Yes
28	220927:3kn	9.0	5.0	0.9	N/A	1.7	-	Yes

1. NC too high
2. TPO efficiency too low and pipetting error
3. Plate dynamic range too low
4. Control did not contain same solvent as test item

Table A3.3. Data for negative control item BP3, corresponding to Figure 8 in the report.

Exp #	Plate ID	Relative inhibition (%)	Standard deviation %
1	211213:1kf	15.6	5.2
2	211215:1kf	-9.0	11.1
3	211216:1kf	3.5	2.4
4	211217:1kf	4.0	8.6
5	220110:1kn	7.2	3.0
6	220202:1kn	3.8	5.8
7	220210:1ap	3.5	0.8
8	220426:1ap	-24.3	9.0
9	220505:1kn	-2.2	6.1
10	220510:1kn	-8.5	9.3
11	220511:1ap	-10.0	2.3
12	220513:1ap	-24.4	2.8
13	220517:1ap	-16.5	4.1
14	220518:1kn	-22.2	12.3
15	220520:1ap	-13.5	14.1
16	220608:1kn	-15.2	5.7
17	220620:1kn	-9.1	3.8
18	220622:1ap	-17.8	3.8
19	220627:1kn	-16.0	3.4
20	220805:1ap	-2.4	3.1
21	220816:1ap	1.7	7.5

Appendix 3

Exp #	Plate ID	Relative inhibition (%)	Standard deviation %
22	220817:1kn	-27.3	11.8
23	220822:1ap	-19.9	5.3
24	220824:1kn	-19.3	5.7
25	220826:1ap	-5.1	4.2
26	220906:1ap	-20.2	10.4
27	220914:1kn	-5.3	4.4
28	220927:1kn	-1.7	9.1

Table A3.4. Data for positive control item PTU corresponding to Figure 9 in the report.

Exp #	Plate ID	Relative inhibition (%)	Standard deviation %
1	211213:1kf	84.0	2.4
2	211215:1kf	78.1	4.6
3	211216:1kf	80.1	0.3
4	211217:1kf	75.7	3.7
5	220110:1kn	76.4	2.8
6	220202:1kn	80.2	1.2
7	220210:1ap	82.4	2.6
8	220426:1ap	67.6	3.4
9	220505:1kn	79.0	4.3
10	220510:1kn	79.2	5.4
11	220511:1ap	77.0	1.2
12	220513:1ap	80.3	0.6
13	220517:1ap	72.7	2.5
14	220518:1kn	83.7	1.8
15	220520:1ap	78.9	0.6
16	220608:1kn	77.3	0.3
17	220620:1kn	92.1	0.4
18	220622:1ap	84.2	2.2
19	220627:1kn	87.5	1.8
20	220805:1ap	86.6	1.6
21	220816:1ap	74.3	3.1
22	220817:1kn	79.6	1.2
23	220822:1ap	90.2	0.7
24	220824:1kn	89.8	1.3
25	220826:1ap	92.2	0.8
26	220906:1ap	80.4	3.4
27	220914:1kn	80.1	1.7
28	220927:1kn	90.1	1.7

Appendix 4

QLI control assay. data for reference and control items

Data for reference item luciferase inhibitor II, negative control item BP3 and positive control item luciferase inhibitor I are presented in tables A4.1-A4.3 below. The data corresponds to figures 10-14 in the report. Multiple experiments were run on the same working solutions the same day if the first experiment failed, each day has its own experiment number. Aberrant numbering of plates is because some plates were not read due to failure.

Table A4.1. Data for acceptance criteria corresponding to figures 10-12 in the report.

Exp #	Plate ID	Plate dynamic range	Z-factor	AC_{50} (µM)	CV (VC) %	Experiment accepted?	Plate accepted?
1	220112:1kn	11484	0.7	3.9E-03	9.9%	No[1,2]	No[1,2]
1	220112:2kn	13216	0.9	5.5E-03	2.1%	Yes	Yes
2	220616:1kn	8001	0.7	5.0E-03	10.3%	No[1,2]	No[1,2]
2	220616:4kn	8595	0.9	7.4E-03	5.0%	Yes	Yes
2	220616:5kn	10402	0.7	N/A	9.0%	-	Yes
2	220616:6kn	8685	0.8	N/A	6.3%	-	Yes
3	220622:3kn	6635	0.9	5.8E-03	3.2%	No[1,2]	No[1,2]
3	220622:4kn	11119	0.9	N/A	3.8%	-	No[3]
3	220622:5kn	12874	0.9	N/A	2.0%	-	No[3]
3	220622:6kn	10031	0.7	6.8E-03	8.7%	No[3]	No[3]
4	220623:1kn	9861	0.7	4.2E-03	9.2%	No[3]	No[3]
4	220623:2kn	11880	0.9	N/A	1.5%	Yes	Yes
4	220623:3kn	12634	1.0	N/A	1.0%	-	Yes
4	220623:4kn	10302	0.9	7.6E-03	2.5%	Yes	Yes
5	220630:1kn	9750	1.0	3.6E-03	1.4%	Yes	Yes
5	220630:2kn	40528	1.0	N/A	1.0%	-	Yes
6	220823:1kn	10258	0.9	5.8E-03	2.9%	Yes	Yes
6	220823:2kn	11545	1.0	N/A	1.1%	-	Yes
7	220829:1kn	10451	1.0	8.5E-03	1.3%	Yes	Yes
7	220829:2kn	10460	0.9	N/A	2.1%	-	Yes
7	220829:2kn	13958	1.0	N/A	0.4%	-	Yes
8	220915:1kn	11268	0.8	3.5E-03	6.2%	No[4]	No[4]
8	220915:2kn	12272	1.0	N/A	1.3%	-	No[4]

1. Standard deviation for controls too high
2. PC too low
3. NC too high
4. Run not valid, NC too high. The result was used anyway, since it was the last round of the control experiment and earlier data was confirmed.

Appendix 4

Table A4.2. Data for negative control item BP3 corresponding to Figure 13 in the report.

Exp #	Plate ID	Relative inhibition (%)	Standard deviation %
1	220112:1kn	13.0	22.7
1	220112:2kn	19.8	0.7
2	220616:1kn	-9.9	35.9
2	220616:4kn	16.3	3.8
3	220622:3kn	12.3	0.9
3	220622:6kn	21.7	1.5
4	220623:1kn	21.4	0.6
4	220623:4kn	19.2	0.6
5	220630:1kn	11.9	3.0
6	220823:1kn	15.6	0.7
7	220829:1kn	11.2	1.1
8	220915:1kn	24.0	0.3

Table A4.3. Data for positive control item luciferase inhibitor I corresponding to Figure 14 in the report.

Exp #	Plate ID	Relative inhibition (%)	Standard deviation %
1	220112:1kn	58.9	61.2
1	220112:2kn	93.8	0.1
2	220616:1kn	46.0	81.2
2	220616:4kn	92.0	0.3
3	220622:3kn	28.9	59.0
3	220622:6kn	95.2	0.1
4	220623:1kn	93.3	0.1
4	220623:4kn	93.6	0.2
5	220630:1kn	93.2	0.1
6	220823:1kn	93.5	0.1
7	220829:1kn	91.9	0.1
8	220915:1kn	94.5	0.2

GETTING IN TOUCH WITH THE EU

In person

All over the European Union there are hundreds of Europe Direct centres. You can find the address of the centre nearest you online (european-union.europa.eu/contact-eu/meet-us_en).

On the phone or in writing

Europe Direct is a service that answers your questions about the European Union. You can contact this service:

— by freephone: 00 800 6 7 8 9 10 11 (certain operators may charge for these calls),

— at the following standard number: +32 22999696,

— via the following form: european-union.europa.eu/contact-eu/write-us_en.

FINDING INFORMATION ABOUT THE EU

Online

Information about the European Union in all the official languages of the EU is available on the Europa website (european-union.europa.eu).

EU publications

You can view or order EU publications at op.europa.eu/en/publications. Multiple copies of free publications can be obtained by contacting Europe Direct or your local documentation centre (european-union.europa.eu/contact-eu/meet-us_en).

EU law and related documents

For access to legal information from the EU, including all EU law since 1951 in all the official language versions, go to EUR-Lex (eur-lex.europa.eu).

Open data from the EU

The portal data.europa.eu provides access to open datasets from the EU institutions, bodies and agencies. These can be downloaded and reused for free, for both commercial and non-commercial purposes. The portal also provides access to a wealth of datasets from European countries.

The European Commission's science and knowledge service

Joint Research Centre

JRC Mission

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

EU Science Hub
joint-research-centre.ec.europa.eu

@EU_ScienceHub

EU Science Hub - Joint Research Centre

EU Science, Research and Innovation

EU Science Hub

EU Science