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Scriptwriter Name: Deepthi Das

Project Page Link: https://review.jove.com/files_upload.php?src=21561413

Title: Identification of Potential Anti-TB Candidates: A Step-by-Step Guide to Synthesis, MIC Determination, and Cytotoxicity Assessment in Mammalian Cells

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **05/20/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 22

Number of Shots: 55

Introduction

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Cristiano Valim Bizarro:** The focus of our research is the development and validation of novel anti-tuberculosis drug candidates aimed at advancing therapeutic strategies.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Cristiano Valim Bizarro:** The presented phenotypic screening pipeline can be used in the search for new pharmaceuticals against *Mycobacterium tuberculosis* strains.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Nicholas Kraether Roth:** This protocol represents a pipeline for the development of drug candidates against tuberculosis based on phenotypic screening.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Nicholas Kraether Roth:** It is important to consider and evaluate the low solubility of the synthesized compounds while applying the protocol.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

1.5. **Nicholas Kraether Roth:** This research can serve as a basis for future bioavailability, macrophage infection and in vivo studies.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Synthesis of 6-Bromo-2-methyl-N-(4-(piperidin-1-yl)phenyl)quinolin-4-amine

Demonstrator: Josiane Delgado da Paz

2.1. To begin, gather the required reagents [1] and proceed with preparing the reaction mixture [2] using the presented proportions [3]. Stir the reaction mixture at 85 degrees Celsius for 16 hours [4]. Use a rotary evaporator to remove the solvent [5].

2.1.1. Talent walking into the room with a tray containing required reagents.

2.1.2. Talent adding reagents into the beaker/flask

2.1.3. **TEXT ON PLAIN BACKGROUND:**

Reaction Mixture:

- 0.53 g (2 mmol) of 6-bromo-4-chloro-2-methylquinoline
- 0.57 g (3 mmol) of 4-(piperidin-1-yl)aniline
- 0.24 mL of pyridine
- 0.20 mL of hydrochloric acid (37%)
- 20 mL of isopropanol (solvent)

2.1.4. Shot of the reaction mixture being stirred at 85 °C.

2.1.5. Shot of the talent using a rotary evaporator to remove the solvent.

2.2. Add approximately 20 milliliters of saturated sodium bicarbonate solution to the residue [1]. Filter this suspension using an 11-micrometer cellulose qualitative filter paper to collect the residual solids [2]. Dissolve the residual solids in 50 milliliters of chloroform [3]. Rinse the chloroform solution with 15 milliliters of water three times, removing the water layer each time [4]. Dry the organic phase using anhydrous sodium sulfate [5].

2.2.1. Talent adding saturated sodium bicarbonate solution to the residue.

2.2.2. Talent filtering the suspension through the 11 µm cellulose filter paper.

2.2.3. Show the talent dissolving the filtered solids in chloroform

2.2.4. Show the talent adding 15 mL water to the chloroform and removing the aqueous layer

2.2.5. Talent adding anhydrous sodium sulfate to dry the organic phase.

2.3. After evaporating the solvent, wash the resulting green solid four times with ten milliliters of hot hexane at approximately 50 degrees Celsius [1]. ~~Perform final purification by flash chromatography on silica gel to get Compound 6 [2-TXT].~~

2.3.1. Shot of the talent washing the green solid with 10 ml hot hexane.

~~2.3.2. Representative shot of the TLC being performed on silica gel with hexane and ethyl acetate as the eluent. **TEXT: Silica gel: 35-70 mesh; Eluent: 1:1 hexane-ethyl acetate**~~ **NOTE: Not filmed, as it is filmed in detail in section 4**

3. Characterization of Compound 6 Using HPLC

Demonstrator: Josiane Delgado da Paz

3.1. Weigh 1 milligram of compound 6 to prepare a stock solution in 1 milliliter acetonitrile-methanol **[1-TXT]**. Dilute the stock solution to 0.5 milligrams per milliliter for analysis **[2]**. Load the sample into the HPLC (*H-P-L-C*) system **[3]**. Use chromatography data acquisition software to acquire and process the data **[4]**.

- 3.1.1. Talent dissolving 1 to 2 milligrams of Compound 6 to acetonitrile-methanol to prepare the stock solution
- 3.1.2. Talent diluting the stock solution for analysis.
- 3.1.3. Talent loading the sample into the high-performance liquid chromatography system.
- 3.1.4. Show the chromatography data acquisition software acquiring and processing the chromatographic data. **NOTE: Filmed using camera**

3.2. Set the chromatographic method to use a 250 by 4.6 millimeters reverse-phase C18 column of particle size 5 micrometers, set the flow rate to 1.5 milliliters per minute, and set UV (*U-V*) detection at 254 nanometers **[1]**. Set up a gradient as presented, with programmed mobile-phase composition changes **[2]**.

- 3.2.1. Chromatography software interface showing the column parameters set to reverse-phase C18 with 5 micrometer particle size and dimensions of 250 by 4.6 millimeters, and then show the flow rate of 1.5 milliliters per minute and UV detection wavelength of 254 nanometers being set in the instrument method settings.
- 3.2.2. TEXT ON A PLAIN BACKGROUND:
Mobile-Phase Gradient Profile
 - 0–7 min: Hold at 100% water with 0.1% acetic acid
 - 7–15 min: Apply linear gradient to 90% acetonitrile–methanol (1:1, v/v)
 - 15–20 min: Return to 100% water with 0.1% acetic acid
 - 20–30 min: Hold at 100% water with 0.1% acetic acid

4. Nuclear Magnetic Resonance (^1H , ^{13}C) Analysis and Purification of Compound 6

Demonstrator: Guilherme Arraché Gonçalves

4.1. Dissolve 15 to 20 milligrams of Compound 6 in DMSO-d₆ (*D-M-S-O-D-Six*) [1]. Transfer the solution into a nuclear magnetic resonance or NMR (*N-M-R*) tube [2].

4.1.1. Talent dissolving 15-20 mg compound 6 in dimethyl sulfoxide-d₆.

4.1.2. Talent transferring the solution into the NMR tube.

4.2. Once the acquisition parameters are set as presented [1], acquire the proton NMR spectrum using a standard pulse sequence [2]. Adjust acquisition parameters, initiate the carbon-13 NMR spectrum, and report chemical shifts in parts per million [3]. Visualize and process the NMR data with the free induction decay file obtained from the experiment [4].

4.2.1. TEXT ON PLAIN BACKGROUND

NMR Acquisition Parameters:

- ¹H NMR: Operate at 400 megahertz
- ¹³C NMR: Operate at 100 megahertz
- Reference standard: Tetramethylsilane

4.2.2. Talent placing the sample into the NMR spectrometer and starting the proton spectral acquisition. *Videographer: Please zoom in to show the screen during acquisition*

4.2.3. Talent adjusting acquisition parameters and initiating the carbon-13 spectral acquisition. Also, show the processed spectrum with chemical shifts displayed in parts per million. **NOTE: Filmed using camera**

4.2.4. Show the free induction decay file being opened to visualize and process the data.

4.3. For chromatography-based purification of compound 6, pack a column with silica gel [1-TXT]. Load the crude compound 6 onto the column [2], elute the compound, and collect fractions [3]. Monitor the fractions using thin-layer chromatography [4] and combine the fractions containing the pure product [5].

4.3.1. Talent preparing and packing the silica gel column.

4.3.2. Talent loading the crude compound 6 onto the column.

4.3.3. Shot of solvent added to the top of column and fractions are collected in the tubes placed in a rack.

4.3.4. Shot of the fractions being separated by thin layer chromatography

4.3.5. Shot of the talent combining thin layer chromatography fractions containing the pure product.

5. Minimal Inhibitory Concentration Determination Using Resazurin Assay

Demonstrator: Marcia Alberton Perelló

- 5.1. Add 100 microliters of 7H9 (7-H-9) broth supplemented with 10 percent Albumin-Dextrose-Catalase or ADC (A-D-C) to column 12 of the 96-well plate [1]. Then transfer 100 microliters of 7H9 broth containing 10 percent ADC and 5 percent DMSO to column 11, and then sequentially from column 9 to 1 [2]. Add 200 microliters of the compound solution to column 10, assigning 1 row per compound [3-TXT].
 - 5.1.1. Talent adding 100 microliters of 7H9 broth supplemented with 10% ADC to column 12 of the 96-well plate.
 - 5.1.2. Shot of talent adding the 7H9 broth containing 10% ADC and 5% dimethyl sulfoxide to columns 11 and 9 through 1.
 - 5.1.3. Talent adding 200 microliters of the compound solution at appropriate concentration to column 10.
- 5.2. Pipette 100 microliters of the compound solution at twice the highest test concentration to column 12 of the 96-well plate [1]. Transfer 100 microliters from column 10 to column 9 and mix well by pipetting to begin a 2-fold serial dilution. Continue the serial dilution sequentially till column 1 [2].
 - 5.2.1. Talent adding 100 microliters of the compound solution to column 12. **NOTE: 5.2.1 and 5.2.2 are filmed in a single shot**
 - 5.2.2. Talent transferring 100 microliters from column 10 to column 9 and mixing by pipetting, and continuing serial dilution till column 1.
Authors: Please demonstrate the process for one full row.
- 5.3. Add 100 microliters of the bacterial suspension of *Mycobacterium tuberculosis* sequentially from column 11 to column 1 and cover the plate [1]. Seal the plate with parafilm and incubate at 37 degrees Celsius for 7 days [2].
 - 5.3.1. Talent adding the bacterial suspension sequentially from columns 11 to column 1.
 - 5.3.2. Shot of the talent sealing the plate and placing it in the incubator.
- 5.4. Next, add 60 microliters of 0.01 percent resazurin solution to each well of the plate and cover it [1]. Seal the plate with parafilm and incubate it at 37 degrees Celsius for 48 hours [2]. Then, check for the color change from blue to pink [1].
 - 5.4.1. Talent adding 60 microliters of 0.01% resazurin solution to each well and covering the plate.
 - 5.4.2. Talent sealing the plate, then placing it in the incubator.
 - 5.4.3. Shot of the plate wells showing the color change from blue to pink.

6. Cytotoxicity Assessment by MTT Assay

Demonstrator: Camili Zanella Zotti

6.1. Prepare a working solution of 0.5 milligram per milliliter MTT (*M-T-T*) in serum-free DMEM (*D-M-E-M*) [1]. **NOTE: The VO is edited for the deleted shot**

6.1.1. Talent preparing the MTT solution in serum-free DMEM.

~~6.1.2. Show the talent filtering the solution through a 0.22 micrometer syringe filter into a tube covered with aluminum foil.~~ **NOTE: Not filmed**

6.2. Obtain Vero and HepG2 (*hep-G-two*) cells treated with Compound 6 for 48 hours and inspect them to assess treatment effects. [1]. Aspirate the medium from the plates [2] and add 50 microliters of the MTT working solution to each well under low ambient light conditions [3].

6.2.1. Shot of the talent checking the cells under a microscope.

Video editor: Please add LAB MEDIA Figure 8a as inset. Please change the label of the top left corner image from 'Untread Control' to 'Untreated Control'

6.2.2. Shot of talent aspirating the medium from the wells.

6.2.3. Add the MTT working solution to it.

6.3. Following a 3-hour incubation, add 100 microliters of DMSO (*D-M-S-O*) to each well to dissolve the formazan crystals, under low ambient light conditions [1]. Place the plate on an orbital shaker at 100 rpm for 15 minutes while keeping it protected from light [2]. Inspect the control wells to confirm complete crystal dissolution [3]. Now, put the plate in a microplate reader, set the reference wavelength of 630 to 690 nanometers, and check the absorbance at 570 nanometers [4].

6.3.1. Talent adding dimethyl sulfoxide to each well.

6.3.2. Talent placing the aluminum foil wrapped plate on an orbital shaker at 100 revolutions per minute for 15 minutes under light protection.

6.3.3. Shot of the talent inspecting the control wells for complete crystal dissolution under the microscope. *Video editor: Please add LAB MEDIA Figure 8b (left panel) as inset and change the top label from 'MTT' to 'Before DMSO'. Alongside them, please add corresponding author provided images and label them 'After DMSO'.* Talent reading the plate on a microplate reader at 570 nanometers with the reference wavelength set to 630 to 690 nanometers.

7. Neutral Red Uptake Assay for Cytotoxicity Assessment

7.1. Prepare a 40 microgram per milliliter neutral red working solution in serum-free DMEM [1]. Then, prepare the neutral red desorption solution containing 1% glacial acetic acid,

49% ethanol, and 50% ultrapure water by volume [2].

7.1.1. Shot of the talent mixing the neutral red solution in serum-free DMEM.

7.1.2. Talent adding glacial acetic acid, ethanol, and ultrapure water to make the neutral red desorption solution.

7.2. Once the effect of Compound 6 is assessed on Vero and HepG2 cells [1], remove the media [2] and add 100 microliters of the filtered neutral red working solution to each well [3]. Incubate the plate for 2 hours at 37 degrees Celsius with 5% carbon dioxide [4].

NOTE: The VO is edited for the additional shot

7.2.1. Reuse 6.2.1

NOTE: Use file "7-2-1-reused-shot-6-2-1.mp4"

Video editor: Please add LAB MEDIA Figure 8a as inset. Please change the label of the top left corner image from 'Untread Control' to 'Untreated Control'.

Added shot: Talent removing the media

7.2.2. Talent adding the neutral red working solution to each well. NOTE: Filmed as "7.2.2-part2, "

7.2.3. Talent placing the plate in the incubator at 37 degrees Celsius with 5% carbon dioxide for 2 hours.

7.3. After removing the neutral red working solution [1], rinse each well once with 150 microliters of sterile PBS [2]. Add 150 microliters of the neutral red desorption solution to each well [3]. Place the plate on an orbital shaker at 150 rpm for 15 minutes to extract the dye [4]. NOTE: The VO is adjusted for the additional shot

ADDED SHOT: Talent removing the media from the well NOTE: slated as 7.3.0

7.3.1. Talent adding 150 microliters of PBS to each well.

7.3.2. Talent adding the neutral red desorption solution to each well.

7.3.3. Talent placing the plate on an orbital shaker at 150 revolutions per minute for 15 minutes. NOTE: Reuse 6.3.2.

7.4. Check the control wells to confirm complete dye extraction [1]. Read the absorbance at 540 nanometers with a microplate reader [2].

7.4.1. Shot of the talent visually inspecting the cells under a microscope.

Video editor: Please add LAB MEDIA Figure 8b (right panel) as inset and change the top label from 'NRU' to 'Before Dye Extraction'. Alongside them, please add corresponding author provided images and label them 'After Dye Extraction'.

7.4.2. Talent reading the plate on a microplate reader at 540 nanometers.

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Results

8. Results

- 8.1. The HPLC chromatogram showed that compound 6 had a retention time of 14.11 minutes [1] and a purity of 99 percent [2].
- 8.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the main peak labeled at 14.11 minutes in the top chromatogram*
- 8.1.2. LAB MEDIA: Figure 4. *Video editor: Highlight the table row showing 99.58 percent in the "Rel.Area %" column corresponding to the 14.11 minutes peak.*
- 8.2. The proton NMR spectrum showed characteristic signals for the piperidine moiety between 1.50 and 3.13 parts per million [1], a methyl singlet at 2.38 parts per million [2], and aromatic signals including doublets at 6.97 and 7.17 parts per million [3] and a multiplet at 7.63 to 7.75 parts per million [4].
- 8.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the peaks between 1.50 and 3.13 on the horizontal scale*
- 8.2.2. LAB MEDIA: Figure 5. *Video editor: Zoom in on the single peak at 2.38*
- 8.2.3. LAB MEDIA: Figure 5. *Video editor: Highlight the peaks at 6.97 and 7.17*
- 8.2.4. LAB MEDIA: Figure 5. *Video editor: Highlight the grouped peaks between 7.63 and 7.75*
- 8.3. The carbon-13 NMR spectrum showed a methyl carbon signal at 23.81 parts per million [1] and piperidine-related signals between 24.85 and 49.70 parts per million [2].
- 8.3.1. LAB MEDIA: Figure 6. *Video editor: Highlight the peak at 23.81*
- 8.3.2. LAB MEDIA: Figure 6. *Video editor: Highlight the peaks between 24.85 and 49.70*
- 8.4. The MTT plate showed a clear color gradient across compound concentrations [1], indicating that cytotoxicity was highest at high compound concentrations with translucent wells [2] and lowest at low compound concentrations with deep purple wells [3].
- 8.4.1. LAB MEDIA: Figure 3B. *Video editor: Show the bottom plate*
- 8.4.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the lighter, more see-through wells on the high-compound-concentration side of the bottom plate*
- 8.4.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the darkest purple wells on the low-compound-concentration side of the bottom plate*

8.5. The neutral red uptake plate showed a clear color gradient across compound concentrations [1], indicating that cytotoxicity was highest at high compound concentrations with translucent wells [2] and lowest at low compound concentrations with deep pink wells [3].

8.5.1. LAB MEDIA: Figure 3B. *Video editor: Show the top plate*

8.5.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the lighter, more see-through wells on the high-compound-concentration side of the top plate*

8.5.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the deepest pink wells on the low-compound-concentration side of the top plate*

1. Quinoline

Pronunciation link: <https://www.merriam-webster.com/dictionary/quinoline>

IPA: /'kwɪnəˌliːn/

Phonetic Spelling: kwin·uh·leen

2. Piperidine

Pronunciation link: <https://www.merriam-webster.com/dictionary/piperidine>

IPA: /pɪˈpɛrɪˌdiːn/

Phonetic Spelling: pi·peh·ruh·deen

3. Pyridine

Pronunciation link: <https://www.merriam-webster.com/dictionary/pyridine>

IPA: /ˈpɪrɪˌdiːn/

Phonetic Spelling: peer·uh·deen

4. Hydrochloric

Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrochloric>

IPA: /ˌhaɪdrəˈklɔːrɪk/

Phonetic Spelling: hy·druh·klor·ik

5. Isopropanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropanol>

IPA: /ˌaɪsəˈproʊpəˌnɔːl/

Phonetic Spelling: eye·suh·proh·puh·nol

6. Rotary evaporator
Pronunciation link: <https://www.merriam-webster.com/dictionary/evaporator>
IPA: /ɪˈvæpəˌreɪtər/
Phonetic Spelling: ih-vap-uh-ray-ter
7. Sodium bicarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/bicarbonate>
IPA: /ˌbaɪˈkɑːrbəˌneɪt/
Phonetic Spelling: by-kaar-buh-nayt
8. Chloroform
Pronunciation link: <https://www.merriam-webster.com/dictionary/chloroform>
IPA: /ˈklɒːrəˌfɔːrm/
Phonetic Spelling: klor-uh-form
9. Anhydrous
Pronunciation link: <https://www.merriam-webster.com/dictionary/anhydrous>
IPA: /ænˈhaɪdrəs/
Phonetic Spelling: an-hy-drus
10. Chromatography
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>
IPA: /ˌkroʊməˈtɑːɡrəfi/
Phonetic Spelling: kroh-muh-taa-gruh-fee
11. Silica
Pronunciation link: <https://www.merriam-webster.com/dictionary/silica>
IPA: /ˈsɪlɪkə/
Phonetic Spelling: सिल-ih-kuh
12. Ethyl acetate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetate>
IPA: /ˈæsəˌteɪt/
Phonetic Spelling: ass-uh-tayt
13. Acetonitrile
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetonitrile>
IPA: /ˌæsətoʊˈnaɪtraɪl/
Phonetic Spelling: ass-uh-toh-nye-tryle
14. Methanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/methanol>
IPA: /ˈmɛθəˌnɔːl/
Phonetic Spelling: meth-uh-nol
15. Chromatographic
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>
IPA: /ˌkroʊməˈtæfɪk/
Phonetic Spelling: kroh-muh-taf-ik
16. Micrometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkrɑːmətər/
Phonetic Spelling: my-krom-uh-ter
17. Nanometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>

- IPA: /'nænə.mi:tər/
Phonetic Spelling: nan·uh·mee·ter
18. Nuclear magnetic resonance
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclear%20magnetic%20resonance>
IPA: /'nu:kliər mæg'netɪk 'rezənəns/
Phonetic Spelling: noo·klee·er mag·net·ik rez·uh·nuhns
19. Tetramethylsilane
Pronunciation link: <https://www.merriam-webster.com/dictionary/tetramethylsilane>
IPA: /,tetrəmə'θɪlsə'leɪn/
Phonetic Spelling: tet·ruh·meth·uhl·suh·layn
20. Mycobacterium tuberculosis
Pronunciation link: <https://www.merriam-webster.com/dictionary/Mycobacterium>
IPA: /,maɪkoʊbæk'tɪriəm/; /tuː'bæ:kjə'loʊsɪs/
Phonetic Spelling: my·koh·bak·teer·ee·um too·ber·kyuh·loh·sis
21. Resazurin
Pronunciation link: <https://www.howtopronounce.com/resazurin>
IPA: /rɪ'sæzjʊrɪn/
Phonetic Spelling: ree·saz·yur·in
22. Cytotoxicity
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytotoxic>
IPA: /,saɪtəʊtɪk'sɪsɪti/
Phonetic Spelling: sy·toh·tok·sis·ih·tee
23. HepG2
Pronunciation link: No confirmed link found
IPA: /hɛp dʒiː tuː/
Phonetic Spelling: hep·gee·two
24. Formazan
Pronunciation link: <https://www.howtopronounce.com/formazan>
IPA: /'fɔːrməzæn/
Phonetic Spelling: for·muh·zan
25. Neutral red
Pronunciation link: <https://www.merriam-webster.com/dictionary/neutral>
IPA: /'nuːtrəl/
Phonetic Spelling: noo·truhl red
26. Glacial acetic acid
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic%20acid>
IPA: /ə'siːtɪk 'æsɪd/
Phonetic Spelling: uh·see·tik ass·id
27. Ultrapure
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrapure>
IPA: /,ʌltrə'pjʊr/
Phonetic Spelling: ul·truh·pyur
28. Orbital shaker
Pronunciation link: <https://www.merriam-webster.com/dictionary/orbital>

IPA: /'ɔ:rbɪtəl/

Phonetic Spelling: or·bih·tuhl

29. Absorbance

Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>

IPA: /əb'zɔ:rbəns/

Phonetic Spelling: ub·zor·buhns

30. Microplate reader

Pronunciation link: <https://www.merriam-webster.com/dictionary/microplate>

IPA: /'maɪkroʊ,pleɪt/

Phonetic Spelling: my·kroh·playt