⟨2251⟩ SCREENING FOR UNDECLARED DRUGS AND DRUG ANALOGUES

INTRODUCTION

The illegal addition of undeclared synthetic compounds to products marketed as dietary supplements[1] (DS) is an issue of universal concern. This fraud is practiced to impart therapeutic effects that cannot be achieved by the dietary ingredients alone. Increasingly, synthetic intermediates and structural analogues of the pharmaceuticals and drugs that have been discontinued or withdrawn from the market are being used as adulterants. Multiple adulterating compounds may be added to a single product, frequently in erratic amounts.

The proposed test methodologies facilitate screening for synthetic adulterants. No individual technique is capable of addressing all potential analytes; thus, a combination of orthogonal approaches adds certainty to the analytical outcome. Mass spectrometric techniques provide strong substantiation of the analytical findings. In some cases, e.g., with hormonal drugs, the amounts of physiologically relevant adulterants may be so low that GC-MS or LC-MS may be the only fitting analytical options.

The express purpose of assembling the procedures recommended herein is their suitability for screening. The level of evidence achievable by application of one or several of the recommended procedures is ultimately dictated by the specific requirements of the end-user. It should be noted that structure elucidation and quantitative assessment are beyond the scope of this chapter.

This chapter is meant to be updated regularly, as new concealment methodologies for the adulterants are introduced, or improvements to the methods of analysis are realized.

ADULTERATION CATEGORIES

The following major categories of adulterated products are recognized:

- **Sexual Enhancement:** This category is also referred to as the Erectile Dysfunction (ED) category. It encompasses a functionally coherent group of adulterants, including several approved drugs, their numerous approved and unapproved analogues, and synthetic intermediates. Their functionality is manifested by inhibition of phosphodiesterase type 5 enzyme (PDE5), which hydrolyzes cyclic guanosine 3′,5′-monophosphate (cGMP); this group of compounds is frequently identified as PDE5 inhibitors. Screening methods for products adulterated with ED compounds are presented in *Appendix A*.
- **Weight Loss (WL):** This category comprises a functionally and chemically diverse collection of compounds that include stimulants, laxatives, diuretics, anorexiants, and psychoactive drugs. Although stimulants constitute an important segment of WL adulterants, the oral anorexiant sibutramine dominates this category, frequently in combination with phenolphthalein, a laxative. Methods for analysis of products adulterated with WL compounds will be addressed in *Appendix B* (to come).
- **Sports Performance Enhancement (SPE):** These compounds constitute the third major category of adulteration. Professional and amateur athletes are targeted with designer anabolic steroids and stimulants, which are systematically banned by the World Anti-Doping Agency. Functional and structural diversity, synthetic proclivity of the adulterators, and the generally small amounts of the infringing substances required to elicit a therapeutic effect make this category especially challenging to address. These supplements are customarily formulated in protein- and fat-rich matrices, thereby further complicating detection. For these reasons, GC- and LC-MS^n techniques constitute primary analytical methodologies within this category. Analysis of products adulterated with SPE compounds will be addressed in *Appendix C* (to come).

BULK INGREDIENTS AND DOSAGE FORMS

Adulteration may occur either at the level of bulk ingredients or at any subsequent stage of the finished product manufacturing. Analysts should be mindful of the possibility of adulterants physically associated with the finished dosage matrix or excipients, as well as components. In the latter, synthetic compounds have been found embedded into the capsule shell body. This underscores the need for deliberate adjustment to the laboratory procedures that typically focus on the capsule contents alone. Appropriate sampling practices for powders and finished dosage forms should be exercised, particularly when only a limited amount of sample is available.

RECOMMENDED ANALYTICAL METHODOLOGIES

Adulteration analysis may be broadly categorized into targeted and nontargeted methods. The distinction between these types may be subtle, and a minor adjustment to the methodology will transform a nontargeted method into a targeted method.

Targeted

These techniques are warranted when the analytes are known. An example of a targeted approach would be monitoring a chromatographic run at a particular wavelength (or mass), and quantifying the analyte that appears within a predefined retention time window. Targeted analysis is conceptually straightforward, because it relies on pre-existing knowledge of the analyte and allows optimization of test methodology for its reliable detection. The targeted approach also is a rarity in the adulterated products analysis, where the nature of the analyte may be anticipated only tentatively, and variable amounts of multiple adulterants belonging to several functional categories are commonplace.

[1] In the United States, dietary supplements are defined as substances that are ingested, in agreement with 21 U.S. Code §321(ff)(2)(A)(i). Definitions of dietary supplements, nutritional supplements, functional foods, and bioactive food additives may vary extensively, depending on local or national legislation. In the marketplace, there is a trend toward expanding the mode of delivery of the adulterating compounds to routes not covered by the regulatory definition for dietary supplements, i.e., topical oils, creams, lotions, e-cigarettes, chewing gums, sprays, and others. Such novel delivery systems present unique challenges, particularly from the standpoint of sample preparation, and are not considered for the purposes of this chapter to be dietary supplements. However, recognizing the emerging threat, USP chooses to highlight the existence of these products. In no way should mention of these products be interpreted as a comment on their legal status or be perceived as an expansion of the definition of DS.

Nontargeted

These methods are better suited to a broad-spectrum detection requirement presented by adulterated products. Nontargeted screening trades precise knowledge of the analyte identity, along with specificity and accuracy, for a wider detection scope. Examples of nontargeted chromatographic screening include acquisition of photodiode array data and full mass-spectral scanning following a chromatographic separation. The procedures in this chapter are written with an eye toward applying all techniques in a nontargeted mode, even the ones considered to be inherently targeted, thereby facilitating detection of a suspect adulterant even in the absence of a matching reference compound.

It is generally recommended to apply a broadly nontargeted methodology first, followed by a targeted procedure. It is crucial to clearly define the end-purpose of analysis, and only then decide on the appropriate instrumentation and assemble a logical testing strategy from the procedures provided. Thus, application of a nontargeted screening method may satisfy the requirements of a manufacturer for the purposes of monitoring bulk raw materials. Conversely, a laboratory requiring a higher level of evidence to enact an enforcement action may opt for a two-step procedure: a preliminary screen, followed by confirmatory analysis of suspect samples.

USP Reference Standards recommended for screening are listed at the end of each relevant *Appendix*. However, considering the rate of propagation of structural analogues and proliferation of newly developed "designer" molecules, establishing and maintaining an all-inclusive catalog of reference materials is both challenging and impractical. Several commercial sources of the compounds of interest exist.[2] Please note that mention of the external reference materials suppliers does not in any way constitute their endorsement, as neither does the listing of reagents, supplies, and instrumentation.

APPENDIX

- **Appendix A. Screening Methodologies for PDE5 Inhibitors**

1. HPLC with Photodiode Array Detection

Solution A: 0.1% Formic acid in water
Solution B: 0.1% Formic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	5	95
23	5	95
24	95	5
31	95	5

Diluent: Acetonitrile and water (50:50)
Standard solution: 100 µg/mL each of USP Sildenafil Citrate RS, USP Tadalafil RS, and USP Vardenafil Hydrochloride RS in *Diluent*
Sample solution: Combine one-fifth of the dosage unit, 10–20 mg of bulk material, or a small fragment of the capsule shell (3 mm × 3 mm) with 10 mL of *Diluent*, sonicate for 30 min, and pass through a 0.2-µm PTFE syringe filter.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: Photodiode array, 200–400 nm
Analytical wavelength: 290 nm
Column: 2.1-mm × 15-cm; 5-µm packing L1[3]
Column temperature: 40°
Flow rate: 0.2 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 1.5
Analysis
Sample: *Sample solution*
Examine the UV spectra of the prominent peaks for similarity to those in the *Standard solution* or other known PDE5 inhibitor compounds (*Figure 1* and *Table 5*). Typical retention times of several PDE5 inhibitors are provided in *Table 5*. However, neither retention time match nor the absorbance spectrum similarity should be construed as sufficient confirmation of the chemical identity of an adulterant.

[2] CacheSyn (http://www.cachesyn.com/); Santa Cruz Biotechnology, Inc. (http://www.scbt.com); TLC Pharmachem (http://www.tlcpharmachem.com/); and Toronto Research Chemicals (http://www.trc-canada.com/) are some of the potential sources of rare and hard-to-find adulterant reference materials.
[3] The procedure was developed on the Agilent Technologies Zorbax SB-C18 column.

2. HPLC with Mass-Spectrometric Detection

Preferably, a mass-spectrometric detector is connected in sequence to the UV-Vis detector. The settings below apply to an ion-trap mass spectrometer. Other MS detectors are suitable; however, it is advisable to use spectrometers that possess MS/MS capability.

Solution A: 0.1% Formic acid in water

Solution B: 0.1% Formic acid in acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	5	95
23	5	95
24	95	5
31	95	5

Diluent: Acetonitrile and water (50:50)

Standard solution: 5 µg/mL each of USP Sildenafil Citrate RS, USP Tadalafil RS, and USP Vardenafil Hydrochloride RS in *Diluent*

Sample solution: Combine one-fifth portion of the dosage unit, 10–20 mg of bulk material, or a small fragment of the capsule shell (3 mm × 3 mm) with 10 mL of *Diluent*, sonicate for 30 min, and pass through the 0.2-µm PTFE syringe filter. Dilute the filtrate 20-fold with *Diluent* before injection.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 2.1-mm × 15-cm; 5-µm packing L1[4]

Column temperature: 40°

Flow rate: 0.2 mL/min

Injection volume: 1 µL

Mass spectrometric system[5]

(See *Mass Spectrometry* ⟨736⟩.)

Ionization: ESI

Polarity: Positive or negative

Sheath gas: 35 mL/min

Sweep gas: 5 mL/min

Capillary temperature: 300°

Source voltage: 5 kV

Collision: 45 meV

Scanning: *m/z* 90–1050 and dependent scan on the most intense ion

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between vardenafil and sildenafil peaks

Tailing factor: NMT 1.5

Analysis

Sample: *Sample solution*

Compare mass-to-charge ratios of the molecular ions $[M+H]^+$ or $[M-H]^-$ and fragments to those of the *Standard solution* or other known analytes listed in *Table 4*. Typical retention times of several common PDE5 inhibitors are provided in *Table 5*.

3. High Performance Thin-Layer Chromatography (HPTLC) with Visual, UV, and/or MS Detection

Standard solution: A composite of 0.2-mg/mL each of USP Sildenafil Citrate RS, USP Tadalafil RS, and USP Vardenafil Hydrochloride RS in methanol, with sonication if necessary. Additional reference materials may be available commercially.

Sample solution: Comminute 1 dosage unit, including the capsule shell and tablet coating, or about 500 mg of raw material; combine with 10 mL of methanol, and subject to ultrasonication for 30 min. Centrifuge or filter the solution, and use the supernatant. [NOTE—Upon development, if the chromatographic bands appear too saturated and UV densitometric spectra are distorted, dilute the *Sample solution* 10-fold with methanol.]

Developing solvent system: *tert*-Butyl methyl ether, methanol, and 28.0% (w/w) ammonium hydroxide (20:2:1). [NOTE—Strength of ammonium hydroxide was found to be crucial for adequate method performance. It is therefore

[4] The procedure was developed on the Agilent Technologies Zorbax SB-C18 column.

[5] The settings were found appropriate for ThermoElectron LTQ XL Linear Ion Trap Mass Spectrometer. Users will need to optimize their respective instrumentation according to the manufacturer's recommendations.

advisable to establish the titer of higher-concentration ammonia[6] and to adjust the latter to exactly 28.0% immediately before the experiment.]

Chromatographic system

(See *HPTLC for Articles of Botanical Origin* ⟨203⟩.)

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm

Application volume: 3 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 47% using a suitable device in the presence of a saturated solution of potassium isothiocyanate.

Temperature: Ambient

Saturation: 20 min, with paper

Developing distance: 6 cm

Derivatization reagent: None

Drying: 5 min in a current of cold air

Detection 1: Visual, under illumination with 254- and 365-nm UV light

Detection 2: UV-Vis spectrometry (scanning densitometer), 190–550 nm

Detection 3: Mass spectrometry, *m/z* 90–1050

Mass spectrometric system[7]

(See *Mass Spectrometry* ⟨736⟩.)

Ionization: ESI

Polarity: Positive, negative, or rapid switching

Desolvation gas (N_2): 300 L/h

Cone gas (N_2): 80 L/h

Temperatures

ESI probe: 105°

Desolvation: 150°

Capillary voltage: 3.0 kV

Cone voltage: 50 V

Scanning: *m/z* 90–1050

System suitability

Sample: *Standard solution*

Suitability requirements: Under UV light at 254 nm, sildenafil, tadalafil, and vardenafil appear as dark bands against the fluorescent background. Under UV light at 365 nm, sildenafil, tadalafil, and vardenafil appear as blue fluorescent bands.

Analysis: Inspect the plate under short-wave (254 nm) and long-wave (365 nm) UV light. PDE5 inhibitors appear as dark bands against the fluorescent background at 254 nm and typically exhibit different shades of blue fluorescence under 365 nm. Note the similarities in R_F values between the bands in the *Standard solution* and *Sample solution*; these may be informative, however they do not constitute sufficient proof of identity. Relative intensities of the bands permit approximation of the amounts. Using scanning densitometry, obtain UV spectra of the prominent bands in the *Sample solution*, and compare them to those of the PDE5 inhibitors in the *Standard solution* and those provided in *Table 5* and *Figure 1*. Mass-spectrometric interface, if available, may facilitate more definitive assignment of the analyte bands: compare mass-to-charge ratios of the molecular ions $[M+H]^+$ or $[M-H]^-$ and fragments to those of the common adulterants listed in *Table 4*.

4. Ambient Ionization Mass Spectrometry

Diluent: Acetonitrile and water (50:50), with 0.1% formic acid

Standard solution: A composite solution containing 20 µg/mL each of USP Sildenafil Citrate RS, USP Tadalafil RS, and USP Vardenafil Hydrochloride RS in *Diluent*. Additional reference materials may be available commercially.

Sample solution: Grind the entire dosage form, including the capsule shell and tablet coating, to a fine powder. Weigh about 50 mg of the resulting powder, or about 50 mg of bulk material, and combine with 5 mL of *Diluent*. Cap tightly, subject to ultrasonication for 2 min, and vortex thoroughly. Centrifuge or filter the resulting solution, and dilute an aliquot of the supernatant or filtrate 100-fold with *Diluent*.

Mass spectrometric system

(See *Applications of Mass Spectrometry* ⟨1736⟩*, Mass Spectrometers, Ionization Procedures, Ambient Ionization Procedures*.)

Ionization: Ambient with thermal desorption

Mode: Thermal profile

Polarity: Positive, negative, or rapid switching

Gas temperature: 150°, 250°, 350°, and 450°

Scanning: *m/z* 90–1050

System suitability: Deposit 3-µL aliquots of the *Standard solution* onto the disposable sample cards. Set the compatible mass spectrometer to a 30-s acquisition of 90–1050 Da. Using one sample card for each temperature setting, acquire mass spectra at each of the following desorption gas temperatures: 150°, 250°, 350°, and 450° in positive ionization mode. Switch polarity, and re-acquire spectra at the same four temperatures in the negative ionization mode. [NOTE—If the mass spectrometer permits rapid polarity switching, both positive and negative spectra may be acquired

[6] 32% Ammonia solution is available from EMD Millipore.

[7] Procedure was developed using Expression CMS mass spectrometer from Advion, equipped with a TLC-MS interface available from CAMAG. If other mass spectrometers are used, relevant settings will have to be optimized. The bands were directly eluted with a mixture of water and acetonitrile (80:20) containing 0.1% formic acid.

simultaneously using a single sample.] Confirm that the $[M+H]^+$ or $[M-H]^-$ ions of sildenafil, tadalafil, and vardenafil are observed as listed in *Table 4*.

Analysis: Deposit 3-µL aliquots of the *Sample solution* onto the disposable sample cards, and follow the procedure outlined above for the *Standard solution*. In the event that a single ion dominates the mass spectrum at every temperature setting, dilute the *Sample solutions* 10-fold with *Diluent* and re-analyze. Compare mass-to-charge ratios of the molecular ions $[M+H]^+$ or $[M-H]^-$ and fragments to those of the known analytes listed in *Table 4*.

5. NMR Spectroscopy–Low-Field and High-Field

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩*, Qualitative and Quantitative NMR Analysis*.) [NOTE—Deuterated acetonitrile (CD_3CN) should be NLT 99.8 atom % D, and should contain 0.05% tetramethylsilane (TMS) as a chemical shift reference. Use of solvents in sealed ampules is recommended. NMR tubes should be suitable for use at the selected magnetic field strength.]

Standard solutions: Dissolve 10 mg of USP Sildenafil Citrate RS, USP Tadalafil RS, or USP Vardenafil Hydrochloride RS in separate 1-mL aliquots of CD_3CN, and transfer 700-µL aliquots of the resulting solutions into individual NMR tubes. Additional reference materials are available commercially.

Sample solution: Grind the entire dosage form, including the capsule shell and tablet coating, to a fine powder. Transfer 100–200 mg of the ground material, or an equivalent amount of bulk raw material powder, into a 5-mL sealable glass vial. Add 1 mL of CD_3CN, vortex thoroughly, and allow the solids to settle. Transfer about 700 µL of the supernatant to an NMR tube, taking care to minimize transfer of solids.

Instrument performance qualification

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)

Magnetic field strength: NLT 42.5 MHz (^1H operating frequency)

Data collection: Use the parameters specified in *Table 3*; perform 90° pulse width calibration before the analysis according to the recommendations of the equipment manufacturer.

Table 3

Parameter	^1H-NMR Qualitative Measurement
Pulse program	Single pulse ^1H
Spectral width	14 ppm (–1 to 13 ppm)
Transmitter offset	Center of spectral width
Relaxation delay	5–10 s
Acquisition time	2–5 s
Number of data points per FID[a]	NLT 16,000
Temperature	25°

[a] Free induction decay.

System suitability: Acquire a ^1H spectrum of the *Standard solution* using the settings outlined in *Data collection*. Record a sufficient number of scans to ensure that signal-to-noise ratio of the TMS signal is NLT 10.

Analysis: Acquire a ^1H spectrum of the *Sample solution* under the conditions outlined in *Data collection*. Record a sufficient number of scans to ensure that the signal-to-noise ratio of the TMS signal is NLT 10. Reference all acquired spectra to the ^1H signal of TMS (0 ppm). Measure and record the chemical shift and multiplicity of the NMR signals in the spectra of the *Standard solutions* and *Sample solution*. Compare the ^1H NMR spectrum of the *Sample solution* to those of the *Standard solutions*, paying particular attention to the aromatic region (5–9 ppm). Determine whether the chemical shift and multiplicity of the NMR signals in the *Sample solution* exhibit sufficient similarity to those found in the *Standard solutions*.

6. Bioassay[8]

PDE5 enzyme[9] **stock solution:** Prepare a concentration of approximately 3000 Units/µL. If necessary, dilute with 40 mM tris(hydroxymethyl)aminomethane hydrochloride (tris-HCl), pH 8.0, 110 mM sodium chloride (NaCl), 2.2 mM potassium chloride (KCl), 3 mM dithiothreitol, and 20% glycerin. Vortex gently to mix.

PDE5 working solution (100 Units per 6.5 µL)**:** Combine 400 µL of PDE-Glo™ Reaction Buffer 5X, 10 µL of *PDE5 enzyme stock solution*, and 1590 µL of water. Vortex gently to mix.

cGMP solution: Combine 400 µL of PDE-Glo™ Reaction Buffer 5X, 40 µL of 1-mM cGMP stock solution, and 1560 µL of water. Mix thoroughly by vortexing.

100-mM IBMX stock solution in DMSO: Prepare a 22.2-mg/mL solution of 3-isobutyl-1-methylxanthine (IBMX) in dimethylsulfoxide (DMSO), e.g., dissolve 100 mg of IBMX in 4.5 mL of DMSO. Mix thoroughly by vortexing.

Reaction buffer: Combine 400 µL of PDE-Glo™ Reaction Buffer 5X and 1600 µL of water. Mix thoroughly by vortexing.

Reaction buffer with 4% DMSO: Combine 400 µL of PDE-Glo™ Reaction Buffer 5X, 80 µL of DMSO and 1520 µL of water. Mix thoroughly by vortexing.

[8] The procedures were developed using commercial Promega PDE-Glo™ Phosphodiesterase Assay Kit, Catalog # V1361. It includes the following reagents: PDE-Glo™ Reaction Buffer 5X (Catalog # V133A); PDE-Glo™ Detection Buffer 5X (Catalog # V134A); Protein Kinase A Solution (Catalog # V135A); PDE-Glo™ Termination Buffer 5X (Catalog # V136A); cGMP Stock Solution, 1 mM (Catalog # V641A); cAMP, 1 mM (Catalog # V642B); Kinase-Glo™ Substrate (Catalog # V672A); and Kinase-Glo™ Buffer (Catalog # V673A). Kits from alternative suppliers may also be used, e.g., BPS Science, Catalog # 60350, although re-optimization of test procedures will be required.

[9] The procedures were developed using human phosphodiesterase 5A from BPS Bioscience Catalog # 60050. The enzyme is available from numerous suppliers, e.g., Sigma-Aldrich Catalog # E9034.

Termination buffer: Combine 400 µL of PDE-Glo™ Termination Buffer 5X, 40 µL of *100-mM IBMX stock solution in DMSO*, and 1560 µL of water. Mix thoroughly by vortexing.

Detection buffer: Combine 400 µL of PDE-Glo™ Detection Buffer 5X, 16 µL of Protein Kinase A Solution, and 1584 µL of water. Vortex gently to mix.

Kinase-Glo™ reagent: Add 10 mL of Kinase-Glo™ Buffer to the vial of Kinase-Glo™ Substrate, and vortex gently.

Standard solution (400 nM)**:** Dissolve 5 mg of USP Sildenafil Citrate RS in 3.0 mL of DMSO to obtain a 2.5-mM stock solution. Combine a 10-µL aliquot of the resulting solution with 240 µL of DMSO, and mix thoroughly (100 µM). Combine a 10-µL aliquot of the resulting solution with 90 µL of DMSO, and mix thoroughly (10 µM). Combine a 10-µL aliquot of the resulting solution with 240 µL of *Reaction buffer*, and mix thoroughly (400 nM); the last dilution is the *Standard solution*.

Control solution: Combine 10 µL of DMSO with 240 µL of *Reaction buffer*, and mix thoroughly by vortexing.

Sample solution: Grind the entire dosage form, including the capsule shell and tablet coating, to a fine powder. Transfer 100 mg of the ground material into a 5-mL polypropylene vial. Add 3.0 mL of DMSO, and vortex for 60 s. Allow solids to settle, combine 50 µL of the clear supernatant with 200 µL of DMSO, and mix thoroughly by vortexing. Combine a 10-µL aliquot of the resulting solution with 90 µL of DMSO, and mix thoroughly by vortexing. Combine a 10-µL aliquot of the resulting solution with 240 µL of *Reaction buffer*, and mix thoroughly by vortexing; the last dilution is the *Sample solution*.

Analysis

1. Dispense 12.5-µL aliquots of the *Standard solution*, *Control solution*, and *Sample solution* into microplate wells, in triplicate. Use a white, flat-bottom, opaque polystyrene, nontreated, 96-well microtiter plate.[10] [NOTE—Do not use treated plates, black plates, or clear plates.]
2. Add 6.5 µL of *PDE5 working solution* to each well. Incubate for 5 min.
3. Add 6.0 µL of *cGMP solution* to effect a 5-µM cGMP concentration in a 25-µL volume. Incubate for 30 min.
4. Add 12.5 µL of *Termination buffer*. Incubate for 5 min.
5. Add 12.5 µL of *Detection buffer*. Incubate for 20 min.
6. Add 50 µL of *Kinase-Glo™ reagent*. Incubate for 10 min.
7. Record luminescence at 560 nm with a microplate luminometer at 0.5 s/well.

[NOTE—Incubate the plate at room temperature, preferably using a plate shaker.]

Calculate average luminescence values for the replicate preparations. Assess the degree of PDE5 inhibition in the *Sample solution* relative to that observed in the *Standard solution* and *Control solution*. Inhibition of PDE5 is manifested as reduction of luminescence: the samples that exhibit suppression of luminescent output comparable to or in excess of that observed with the *Standard solution* are likely adulterated with synthetic PDE5 inhibitors.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sildenafil Citrate RS
USP Tadalafil RS
USP Vardenafil Hydrochloride RS

Table 4. Mass Spectral Data for Select PDE5 Inhibitors[a]

#	Name	CAS Number	Chemical Formula	Exact Mass	$[M+H]^+$	$[M-H]^-$	Fragments
1	Acetaminotadalafil	1446144-71-3	$C_{23}H_{20}N_4O_5$	432.1434	433	—	455 $[M+Na]^+$, 391, 311, 269, 250
2	Acetil acid	147676-78-6	$C_{18}H_{20}N_4O_4$	356.1485	357	—	329, 300, 285, 268, 256, 242, 166, 131
3	Acetildenafil (Hongdenafil)	831217-01-7	$C_{25}H_{34}N_6O_3$	466.26924	467.28	—	449, 439, 420, 404, 396, 381, 355, 353, 339, 325, 324, 311, 297, 285, 166, 127, 111, 99, 97
4	Acetylvardenafil	1261351-28-3	$C_{25}H_{34}N_6O_3$	466.2692	467	—	439, 396, 341, 317, 270
5	Aildenafil (Dimethylsildenafil, Methisosildenafil)	496835-35-9	$C_{23}H_{32}N_6O_4S$	488.22057	489.23 —	— 487.40	432, 377, 313, 311, 283, 113, 99 460, 310, 282
6	Aminotadalafil	385769-84-6	$C_{21}H_{18}N_4O_4$	390.1328	391.14 —	— 389.1248	269, 262, 241, 239, 224, 197, 169 362, 298, 262, 234, 233, 232
7	(*S,R*)-Aminotadalafil [(+)-*trans*-Aminotadalafil]	1093940-70-5	$C_{21}H_{18}N_4O_4$	390.1328	391	—	No data
8	Avanafil	330784-47-9	$C_{23}H_{26}N_7O_3Cl$	483.1786	484.186	—	375, 349, 221

[10] Conforming plates are available from Corning (Costar 3912 or Costar 3963), Thermo Scientific (Nunc™ Catalog #236105), and other vendors.

Table 4. Mass Spectral Data for Select PDE5 Inhibitors[a] *(continued)*

#	Name	CAS Number	Chemical Formula	Exact Mass	$[M+H]^+$	$[M-H]^-$	Fragments
9	Benzylsildenafil	1446089-82-2	$C_{28}H_{34}N_6O_4S$	550.2362	551	—	377, 283
10	*N*-Butylnortadalafil	171596-31-9	$C_{25}H_{25}N_3O_4$	431.18451	432.25	—	**310**,[b] *282,*[c] *197, 169*
11	Carbodenafil (Fondenafil)	944241-52-5	$C_{24}H_{32}N_6O_3$	452.2536	453	—	283
12	Chlorodenafil	1058653-74-9	$C_{19}H_{21}ClN_4O_3$	388.8485	390	—	360, 311, 291, 254, 183, 136
13	Chloropretadalafil	171489-59-1	$C_{22}H_{19}ClN_2O_5$	426.0982	427	—	429, 395, 349, 334, 302, 287, 262, 229, 159, 135
14	Cinnamyldenafil	1446089-83-3	$C_{32}H_{38}N_6O_3$	554.3005	554	—	488, 354, 297, 283, 215, 166, 117, 91
15	Cyclopentynafil	1173706-34-7	$C_{26}H_{36}N_6O_4S$	528.2519	529	—	461, 153
16	Depiperazinothiosildenafil	1353018-10-6	$C_{17}H_{20}N_4O_4S_2$	408.0926	409 —	— 407.0845	381, 351, 327, 299, 285, 272 378, 314
17	Descarbonsildenafil	1393816-99-3	$C_{21}H_{30}N_6O_4S$	462.2049	463	—	418, 377, 360, 311, 299, 283, 255, 151, 87
18	*N*-Desethylvardenafil	448184-46-1	$C_{21}H_{28}N_6O_4S$	460.18927	461.20	—	392, 377, 376, 329, 313, 312, 299, 284, 283, 151
19	Desmethylcarbodenafil	147676-79-7	$C_{23}H_{31}N_6O_3$	438.523	439.2451	—	339, 311
20	*N*-Desmethylsildenafil	139755-82-1	$C_{21}H_{28}N_6O_4S$	460.18927	461.19	—	377, 313, 311, 299, 283
21	Dimethylacetildenafil	1417999-76-8	$C_{25}H_{34}O_3N_6$	466.2771	467	—	279, 149, 177
22	Dioxo-acetildenafil	1609405-33-5	$C_{25}H_{30}N_6O_5$	494.2278	495	—	No data
23	Dithio-desmethylcarbodenafil	1333233-46-7	$C_{23}H_{30}N_6OS_2$	470.6572	471.1991	—	371, 343
24	Gendenafil	147676-66-2	$C_{19}H_{22}N_4O_3$	354.16919	355.31	—	327, 298, **285**, 283, *256, 242*
25	Gisadenafil	334826-98-1	$C_{23}H_{33}N_7O_5S$	519.22639	520	—	No data
26	Homosildenafil	642928-07-2	$C_{23}H_{32}N_6O_4S$	488.22057	489.23	—	467, 461, 377, 313, 311, 283, 127, 111, 113, 99, 97
27	Hydroxyacetildenafil (Hydroxyhongdenafil)	147676-56-0	$C_{25}H_{34}N_6O_4$	482.26415	483.27	—	465, 447, 439, 396, 353, 339, 325, 311, 297
28	Hydroxychlorodenafil	1391054-00-4	$C_{19}H_{23}ClN_4O_3$	390.1459	391	—	No data
29	Hydroxyhomosildenafil	139755-85-4	$C_{23}H_{32}N_6O_5S$	504.21549	505.22 —	— 503.31	487, 461, 423, 377, 312, 311, 283, 284, 225, 166, 129, 112, 99, 97 475, 310, 282
30	2-Hydroxypropylnortadalafil	1353020-85-5	$C_{24}H_{23}N_3O_5$	433.16377	434.15	—	**312**, *284, 197, 169*
31	Hydroxythiohomosildenafil (Hydroxyhomosildenafil thione, Sulfohydroxyhomosildenafil)	479073-82-0	$C_{23}H_{32}N_6O_4S_2$	520.19264	521.20	—	503, 477, 461, 419, 393, 355, 354, 327, 325, 291
32	Hydroxythiovardenafil	912576-30-8	$C_{23}H_{32}N_6O_4S_2$	520.1926	521	—	No data
33	Hydroxyvardenafil	224785-98-2	$C_{23}H_{32}N_6O_5S$	504.2155	505.26 —	— 503	344, 312, 253, 169, 99 475, 310, 282
34	Imidazosagatriazinone (Desulfovardenafil)	139756-21-1	$C_{17}H_{20}N_4O_2$	312.1586	313	—	284, 256, 169, 151
35	Isopiperazinonafil	1335201-06-3	$C_{25}H_{34}N_6O_4$	482.2642	483 —	— 481	— 453, 422, 379, 336, 325, 311, 309

Table 4. Mass Spectral Data for Select PDE5 Inhibitors[a] *(continued)*

#	Name	CAS Number	Chemical Formula	Exact Mass	$[M+H]^+$	$[M-H]^-$	Fragments
36	Lodenafil carbonate	398507-55-6	$C_{47}H_{62}N_{12}O_{11}S_2$	1034.4102	1035	—	518, **487**, *377, 311*
37	Mirodenafil	862189-95-5	$C_{26}H_{37}N_5O_5S$	531.6698	532	—	488, 404, 362, 296, 268
38	Mutaprodenafil	1387577-30-1	$C_{27}H_{35}N_9O_5S_2$	629.7635	630.2279	—	489, 377, 142, 113
39	Nitrodenafil	147676-99-1	$C_{17}H_{19}N_5O_4$	357.3647	358	—	307, 289, 261, 217, 176, 154, 136, 107, 89
40	Nitroso-prodenafil	1266755-08-1	$C_{27}H_{35}N_9O_5S_2$	629.2203	630	—	142
41	Noracetildenafil (Demethylhongdenafil)	949091-38-7	$C_{24}H_{32}N_6O_3$	452.25359	453.26	—	425, 406, 396, 380, 367, 355, 353, 339, 325, 324, 313, 297, 296, 253
42	Norneosildenafil (Piperidino sildenafil)	371959-09-0	$C_{22}H_{29}N_5O_4S$	459.19403	460.20	—	432, 377, 329, 311, 299, 283
43	Norneovardenafil	358390-39-3	$C_{18}H_{20}N_4O_4$	356.1485	357	—	329, 307, 289, 176, 154, 136, 107, 99
44	Nortadalafil (Demethyltadalafil)	171596-36-4	$C_{21}H_{17}N_3O_4$	375.1219	376	374.1138	262, 234, 233, 232
45	*N*-Octylnortadalafil (Octylnortadalafil)	1173706-35-8	$C_{29}H_{33}N_3O_4$	487.2471	488	—	366, 227
46	Oxohongdenafil	1446144-70-2	$C_{25}H_{32}N_6O_4$	480.2485	481	—	451, 396, 354, 339, 312, 297, 289
47	Piperazinonafil (Piperazonifil, Dihydroacetildenafil)	1335201-04-1	$C_{25}H_{34}N_6O_4$	482.2642	483 —	— 481	— 453, 435, 348, 336, 321, 311, 309, 282, 267
48	Piperidino acetildenafil (Piperiacetildenafil)	147676-50-4	$C_{24}H_{31}N_5O_3$	437.2427	438	—	410, 408, 355, 353, 341, 325, 297, 288
49	Piperidinovardenafil (Piperidenafil, Pseudovardenafil)	224788-34-5	$C_{22}H_{29}N_5O_4S$	459.19403	460.20	—	432, 403, 391, 377, 349, 329, 312, 311, 301, 299, 284, 283, 270, 256, 169, 151
50	Propoxyphenyl aildenafil	1391053-82-9	$C_{24}H_{34}N_6O_4S$	502.2362	503	—	252
51	Propoxyphenyl hydroxyhomosildenafil (Methylhydroxyhomosildenafil)	139755-87-6	$C_{24}H_{34}N_6O_5S$	518.2311	519	—	501, 475, 391, 331, 325, 299, 283, 129, 112, 99
52	Propoxyphenyl sildenafil	877777-10-1	$C_{23}H_{32}N_6O_4S$	488.2205	489.2272	—	447, 391, 325, 299, 283, 100
53	Propoxyphenyl thioaildenafil (Propoxyphenyl thiomethisosildenafil)	856190-49-3	$C_{24}H_{34}N_6O_3S_2$	518.2133	519	—	260
54	Propoxyphenyl thiohydroxyhomosildenafil	479073-90-0	$C_{24}H_{34}N_6O_4S_2$	534.2083	535.2150	—	517, 359, 341, 315, 299, 271, 129, 112, 99
55	Sildenafil	139755-83-2	$C_{22}H_{30}N_6O_4S$	474.20492	475.21 —	— 473.45	447, 418, 391, 377, 374, 346, 329, 311, 297, 283, 255, 163, 160, 100 445, 310, 282
56	Tadalafil (Tildenafil)	171596-29-5	$C_{22}H_{19}N_3O_4$	389.13756	390 —	— 388.1288	302, 268, 262, 250, 240, 197, 169, 135 262, 234, 233, 232
57	(–)-*trans*-Tadalafil (ent-Tadalafil)	629652-72-8	$C_{22}H_{19}N_3O_4$	389.1376	390	—	779 $[2M+H]^+$, 262, 250, 135
58	Thioaildenafil (Sulfoaildenafil, Thiomethisosildenafil, Sulfodimethyl sildenafil, Dimethylthiosildenafil)	856190-47-1	$C_{23}H_{32}N_6O_3S_2$	504.19773	505.21	—	448, 393, 327, 299, 113, 99

Table 4. Mass Spectral Data for Select PDE5 Inhibitors[a] *(continued)*

#	Name	CAS Number	Chemical Formula	Exact Mass	$[M+H]^+$	$[M-H]^-$	Fragments
59	Thiohomosildenafil (Sulfohomosildenafil, Homosildenafil thione)	479073-80-8	$C_{23}H_{32}N_6O_3S_2$	504.19773	505.21	—	477, 421, 393, 357, 355, 343, 327, 315, 299, 271, 113, 99
60	Thioquinapiperifil (KF31327)	220060-39-9	$C_{24}H_{28}N_6OS$	448.2045	449	—	363, 246, 225, 204, 121
61	Thiosildenafil (Sulfosildenafil, Sildenafil thione)	479073-79-5	$C_{22}H_{30}N_6O_3S_2$	490.18208	491.19	—	407, 393, 343, 341, 327, 315, 313, 299, 283, 271, 163, 99
62	Udenafil	268203-93-6	$C_{25}H_{36}N_6O_4S$	516.2519	517.260	—	474, 418, 347, 325, *299*, 283
63	Vardenafil	224785-90-4	$C_{23}H_{32}N_6O_4S$	488.22057	489.2274 —	— 487.33	461, 420, 377, 376, 375, 346, 339, 329, 312, 299, 284, 283, 169, 151, 123, 99 459, 310, 282
64	Xanthoanthrafil (Benzamidenafil)	1020251-53-9	$C_{19}H_{23}N_3O_6$	389.15869	390.31	—	344, 252, 223, **151**, *107, 91*

[a] Compiled from peer-reviewed literature, and communications with USP collaborators. See corresponding chemical structures in *Figure 2*.
[b] **Bold**, fragment subjected to MS^3 fragmentation.
[c] *Italics*, MS^3 fragments derived from the parent fragment (**bold**).

Table 5. UV Absorbance Maxima and Retention Time Data for Select PDE5 Inhibitors[a]

#	Name	CAS Number	Chemical Formula	UV Absorbance Maxima (nm)	UV Absorbance Spectrum Type (*Figure 1*)	Retention Time (min)[b]	Relative Retention Time with Respect to Sildenafil
1	Acetaminotadalafil	1446144-71-3	$C_{23}H_{20}N_4O_5$	202, 222, 282	b	14.3	1.1
2	Acetil acid	147676-78-6	$C_{18}H_{20}N_4O_4$	230, 260, 285	—	—	—
3	Acetildenafil (Hongdenafil)	831217-01-7	$C_{25}H_{34}N_6O_3$	234, 282	e	12.5	1.0
4	Acetylvardenafil	1261351-28-3	$C_{25}H_{34}N_6O_3$	218, 246, 268(s)[c]	d	11.4	0.9
5	Aildenafil (Dimethylsildenafil, Methisosildenafil)	496835-35-9	$C_{23}H_{32}N_6O_4S$	226, 294	a	13.3	1.0
6	Aminotadalafil	385769-84-6	$C_{21}H_{18}N_4O_4$	200, 220, 284, 290(s)	b	14.2	1.1
7	(*S,R*)-Aminotadalafil [(+)-*trans*-Aminotadalafil]	1093940-70-5	$C_{21}H_{18}N_4O_4$	225, 283	—	—	—
8	Avanafil	330784-47-9	$C_{23}H_{26}N_7O_3Cl$	198, 244	f	13.0	1.0
9	Benzylsildenafil	1446089-82-2	$C_{28}H_{34}N_6O_4S$	291	—	—	—
10	*N*-Butylnortadalafil	171596-31-9	$C_{25}H_{25}N_3O_4$	222, 284	—	—	—
11	Carbodenafil (Fondenafil)	944241-52-5	$C_{24}H_{32}N_6O_3$	295	—	—	—
12	Chlorodenafil	1058653-74-9	$C_{19}H_{21}ClN_4O_3$	211, 235, 279	—	—	—
13	Chloropretadalafil	171489-59-1	$C_{22}H_{19}ClN_2O_5$	204, 222, 284	b	17.4	1.3
14	Cinnamyldenafil	1446089-83-3	$C_{32}H_{38}N_6O_3$	239	—	—	—
15	Cyclopentynafil	1173706-34-7	$C_{26}H_{36}N_6O_4S$	218, 290	—	—	—
16	Depiperazinothiosildenafil	1353018-10-6	$C_{17}H_{20}N_4O_4S_2$	295, 354	—	—	—
17	Descarbonsildenafil	1393816-99-3	$C_{21}H_{30}N_6O_4S$	225, 295	—	—	—
18	*N*-Desethylvardenafil	448184-46-1	$C_{21}H_{28}N_6O_4S$	226, 246(s)	d	11.9	0.9
19	Desmethylcarbodenafil	147676-79-7	$C_{23}H_{31}N_6O_3$	226,296	a	11.9	0.9
20	*N*-Desmethylsildenafil	139755-82-1	$C_{21}H_{28}N_6O_4S$	224, 294	a	12.9	1.0
21	Dimethylacetildenafil	1417999-76-8	$C_{25}H_{34}O_3N_6$	233, 276	—	—	—
22	Dioxo-acetildenafil	1609405-33-5	$C_{25}H_{30}N_6O_5$	No data	—	—	—

Table 5. UV Absorbance Maxima and Retention Time Data for Select PDE5 Inhibitors[a] *(continued)*

#	Name	CAS Number	Chemical Formula	UV Absorbance Maxima (nm)	UV Absorbance Spectrum Type (*Figure 1*)	Retention Time (min)[b]	Relative Retention Time with Respect to Sildenafil
23	Dithio-desmethylcarbodenafil	1333233-46-7	$C_{23}H_{30}N_6OS_2$	258, 285, 356	—	—	—
24	Gendenafil	147676-66-2	$C_{19}H_{22}N_4O_3$	232, 274	f	16.8	1.3
25	Gisadenafil	334826-98-1	$C_{23}H_{33}N_7O_5S$	No data	—	—	—
26	Homosildenafil	642928-07-2	$C_{23}H_{32}N_6O_4S$	226, 292	a	13.3	1.0
27	Hydroxyacetildenafil (Hydroxyhongdenafil)	147676-56-0	$C_{25}H_{34}N_6O_4$	234, 280	e	12.2	0.9
28	Hydroxychlorodenafil	1391054-00-4	$C_{19}H_{23}ClN_4O_3$	212, 303	—	—	—
29	Hydroxyhomosildenafil	139755-85-4	$C_{23}H_{32}N_6O_5S$	226, 296	a	12.9	1.0
30	2-Hydroxypropylnortadalafil	1353020-85-5	$C_{24}H_{23}N_3O_5$	222, 284	—	—	—
31	Hydroxythiohomosildenafil (Hydroxyhomosildenafil thione, Sulfohydroxyhomosildenafil)	479073-82-0	$C_{23}H_{32}N_6O_4S_2$	228, 296, 352	c	15.0	1.2
32	Hydroxythiovardenafil	912576-30-8	$C_{23}H_{32}N_6O_4S_2$	203, 235, 316	—	—	—
33	Hydroxyvardenafil	224785-98-2	$C_{23}H_{32}N_6O_5S$	216	—	—	—
34	Imidazosagatriazinone (Desulfovardenafil)	139756-21-1	$C_{17}H_{20}N_4O_2$	212, 253	—	—	—
35	Isopiperazinonafil	1335201-06-3	$C_{25}H_{34}N_6O_4$	221, 290	—	—	—
36	Lodenafil carbonate	398507-55-6	$C_{47}H_{62}N_{12}O_{11}S_2$	226, 296	a	15.4	1.2
37	Mirodenafil	862189-95-5	$C_{26}H_{37}N_5O_5S$	216, 248	f	14.5	1.1
38	Mutaprodenafil	1387577-30-1	$C_{27}H_{35}N_9O_5S_2$	218, 240, 283, 297, 335	—	—	—
39	Nitrodenafil	147676-99-1	$C_{17}H_{19}N_5O_4$	212, 298	—	—	—
40	Nitroso-prodenafil	1266755-08-1	$C_{27}H_{35}N_9O_5S_2$	241, 301	—	—	—
41	Noracetildenafil (Demethylhongdenafil)	949091-38-7	$C_{24}H_{32}N_6O_3$	234, 280	e	12.3	0.9
42	Norneosildenafil (Piperidino sildenafil)	371959-09-0	$C_{22}H_{29}N_5O_4S$	226, 300	a	18.7	1.4
43	Norneovardenafil	358390-39-3	$C_{18}H_{20}N_4O_4$	215, 241	—	—	—
44	Nortadalafil (Demethyltadalafil)	171596-36-4	$C_{21}H_{17}N_3O_4$	No data	—	—	—
45	*N*-Octylnortadalafil (Octylnortadalafil)	1173706-35-8	$C_{29}H_{33}N_3O_4$	281	—	—	—
46	Oxohongdenafil	1446144-70-2	$C_{25}H_{32}N_6O_4$	481	—	—	—
47	Piperazinonafil (Piperazonifil, Dihydroacetildenafil)	1335201-04-1	$C_{25}H_{34}N_6O_4$	221, 290	—	—	—
48	Piperidino acetildenafil (Piperiacetildenafil)	147676-50-4	$C_{24}H_{31}N_5O_3$	234, 284	e	13.1	1.0
49	Piperidinovardenafil (Piperidenafil, Pseudovardenafil)	224788-34-5	$C_{22}H_{29}N_5O_4S$	224, 246(s)	d	16.5	1.3
50	Propoxyphenyl aildenafil	1391053-82-9	$C_{24}H_{34}N_6O_4S$	215, 225, 295	—	—	—
51	Propoxyphenyl hydroxyhomosildenafil (Methylhydroxyhomosildenafil)	139755-87-6	$C_{24}H_{34}N_6O_5S$	226, 294	a	13.4	1.0
52	Propoxyphenyl sildenafil	877777-10-1	$C_{23}H_{32}N_6O_4S$	226, 292	a	13.6	1.0
53	Propoxyphenyl thioaildenafil (Propoxyphenyl thiomethisosildenafil)	856190-49-3	$C_{24}H_{34}N_6O_3S_2$	227, 295, 355	—	—	—
54	Propoxyphenyl thiohydroxyhomosildenafil	479073-90-0	$C_{24}H_{34}N_6O_4S_2$	227, 295, 353	—	—	—

Table 5. UV Absorbance Maxima and Retention Time Data for Select PDE5 Inhibitors[a] *(continued)*

#	Name	CAS Number	Chemical Formula	UV Absorbance Maxima (nm)	UV Absorbance Spectrum Type (*Figure 1*)	Retention Time (min)[b]	Relative Retention Time with Respect to Sildenafil
55	Sildenafil	139755-83-2	$C_{22}H_{30}N_6O_4S$	224, 294	a	13.0	1.0
56	Tadalafil (Tildenafil)	171596-29-5	$C_{22}H_{19}N_3O_4$	200, 222, 284, 292(s)	b	15.0	1.2
57	(–)-*trans*-Tadalafil (ent-Tadalafil)	629652-72-8	$C_{22}H_{19}N_3O_4$	231, 282, 289	—	—	—
58	Thioaildenafil (Sulfoaildenafil, Thiomethisosildenafil, Sulfodimethyl sildenafil, Dimethylthiosildenafil)	856190-47-1	$C_{23}H_{32}N_6O_3S_2$	228, 250(s), 296, 352, 366(s)	c	15.5	1.2
59	Thiohomosildenafil (Sulfohomosildenafil, Homosildenafil thione)	479073-80-8	$C_{23}H_{32}N_6O_3S_2$	228, 248(s), 296, 354, 370(s)	c	14.4	1.1
60	Thioquinapiperifil (KF31327)	220060-39-9	$C_{24}H_{28}N_6OS$	211, 268, 363	—	—	—
61	Thiosildenafil (Sulfosildenafil, Sildenafil thione)	479073-79-5	$C_{22}H_{30}N_6O_3S_2$	228, 250(s), 296, 356, 368(s)	c	15.2	1.2
62	Udenafil	268203-93-6	$C_{25}H_{36}N_6O_4S$	228, 298	f	13.6	1.0
63	Vardenafil	224785-90-4	$C_{23}H_{32}N_6O_4S$	226, 252(s)	d	12.1	0.9
64	Xanthoanthrafil (Benzamidenafil)	1020251-53-9	$C_{19}H_{23}N_3O_6$	202, 228, 278, 390	f	15.3	1.2

[a] Compiled from peer-reviewed literature, and contributed by the USP collaborators. See corresponding chemical structures in *Figure 2*.
[b] Retention times derived from the experiments conducted as described in *HPLC with Photodiode Array Detection* and in *HPLC with Mass-Spectrometric Detection* methods.
[c] (s) denotes a shoulder.

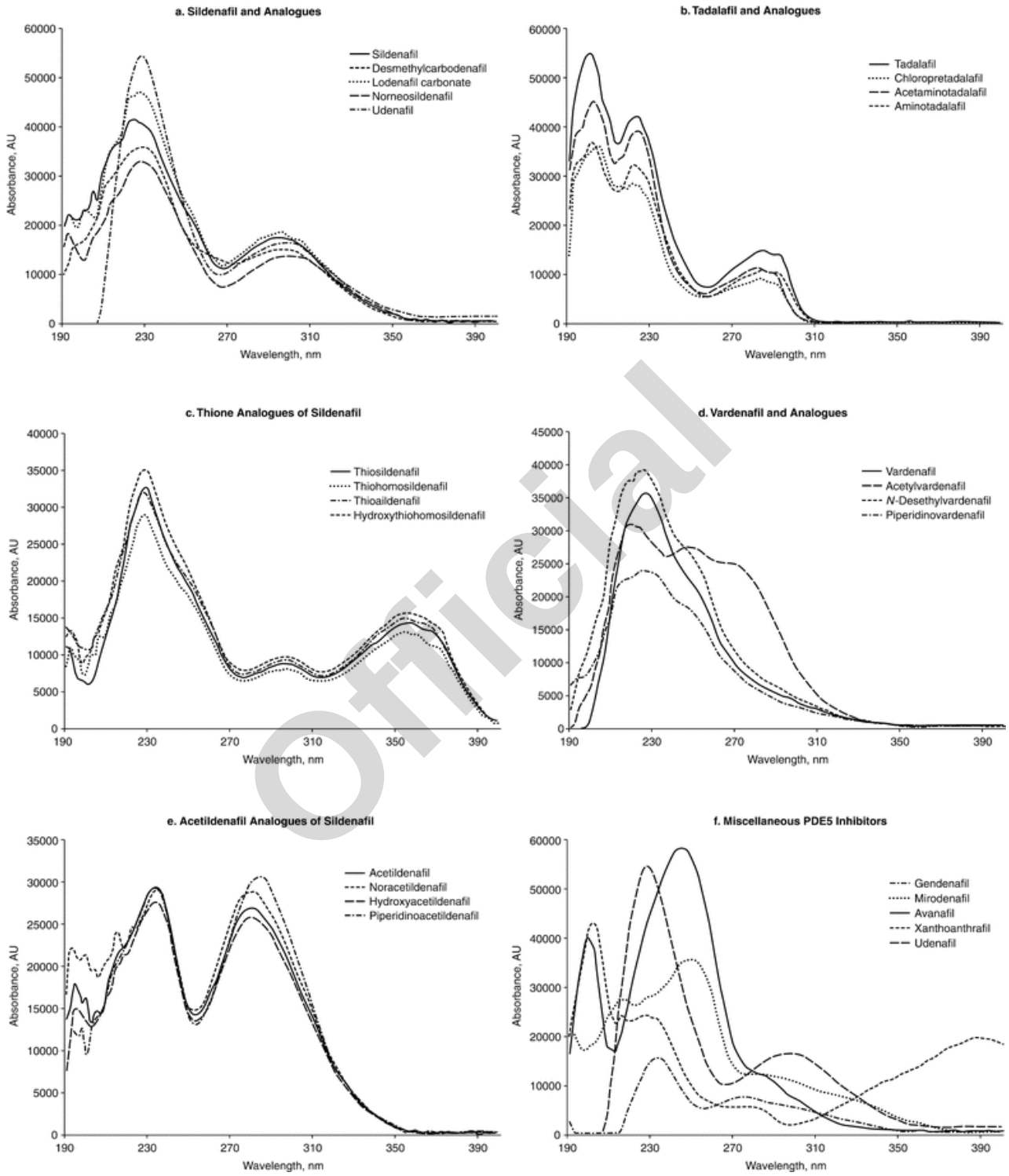
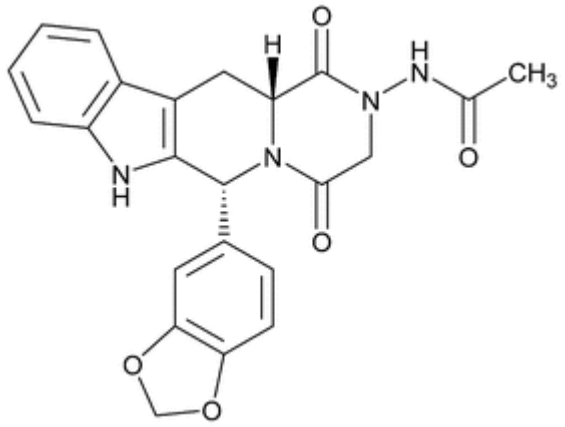


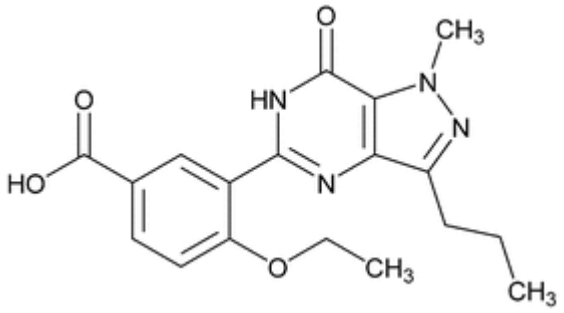
Figure 1. UV absorbance spectra of select PDE5 inhibitors.[11]

[11] Data contributed by USP collaborators.

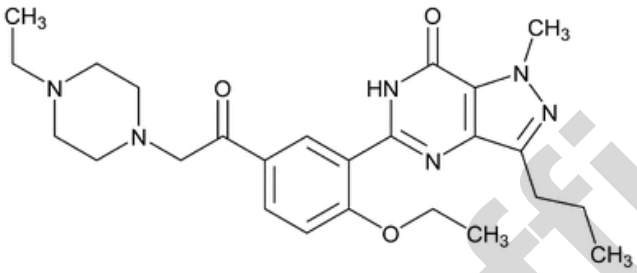
Figure 2. Chemical structures of select PDE5 inhibitors.[12]



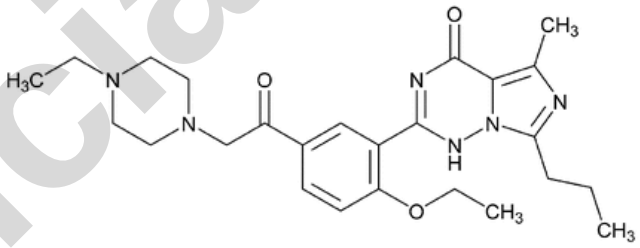
Acetaminotadalafil



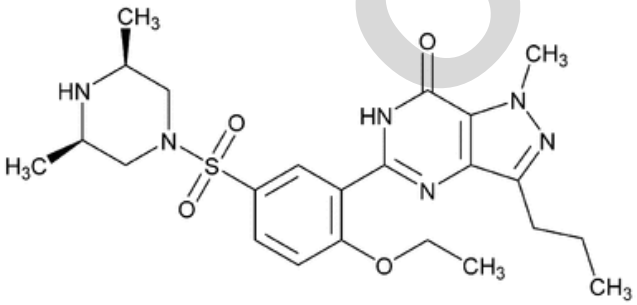
Acetil acid



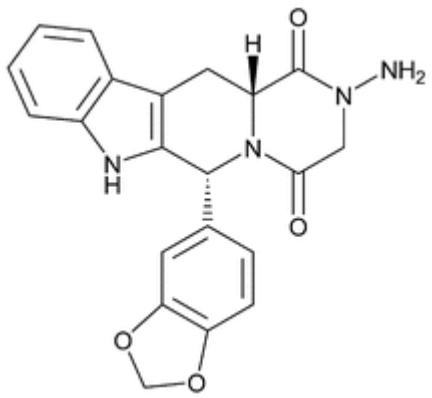
Acetildenafil



Acetylvardenafil

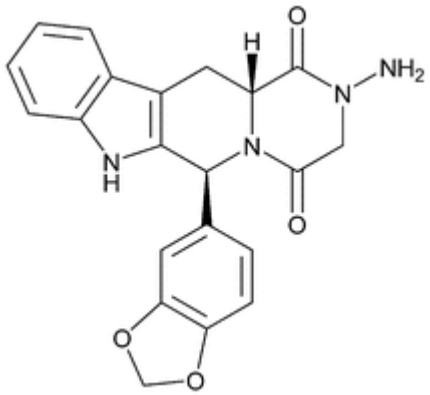


Aildenafil

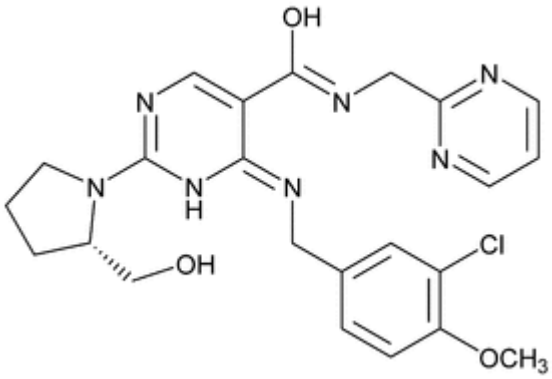


Aminotadalafil

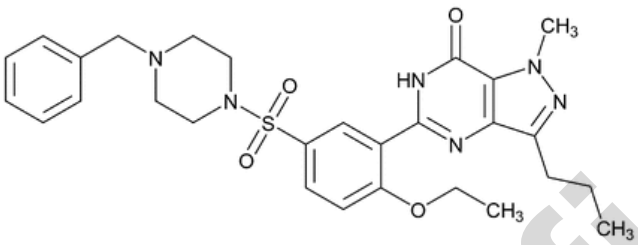
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



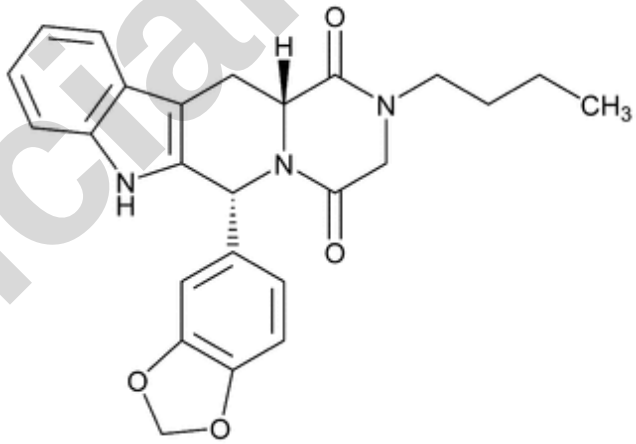
(*S,R*)-Aminotadalafil



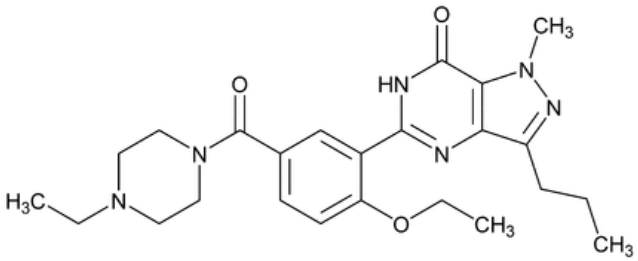
Avanafil



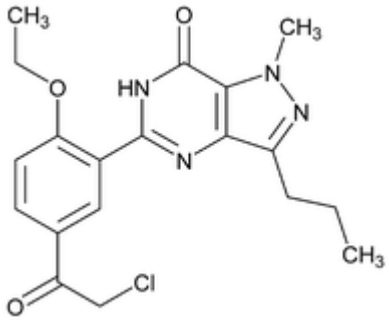
Benzylsildenafil



N-Butylnortadalafil

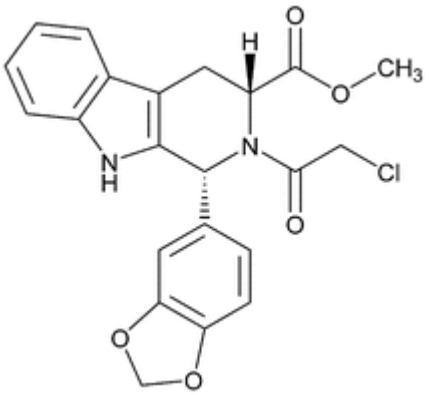


Carbodenafil

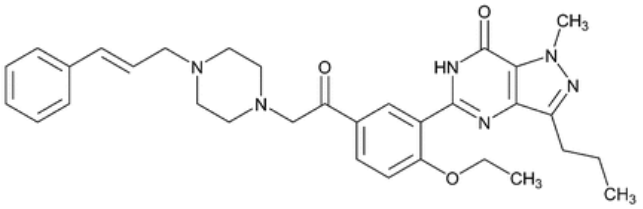


Chlorodenafil

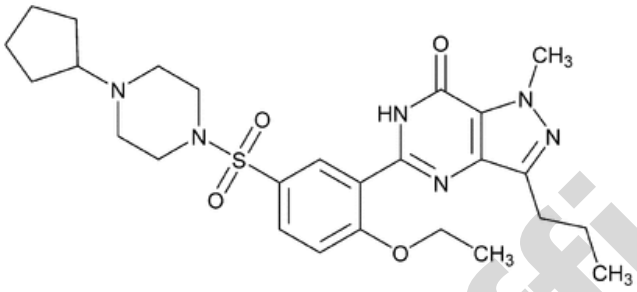
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



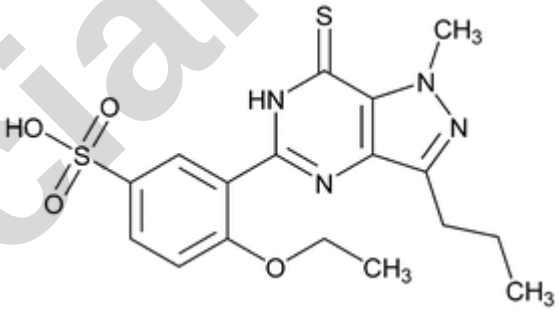
Chloropretadalafil



Cinnamyldenafil



Cyclopentynafil



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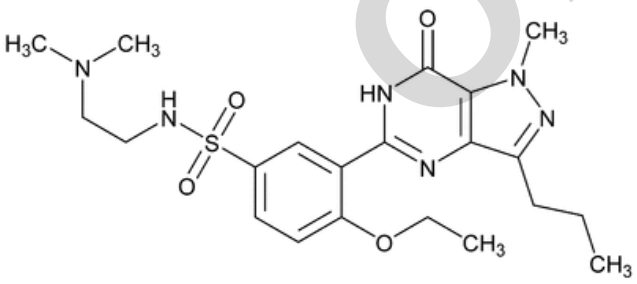

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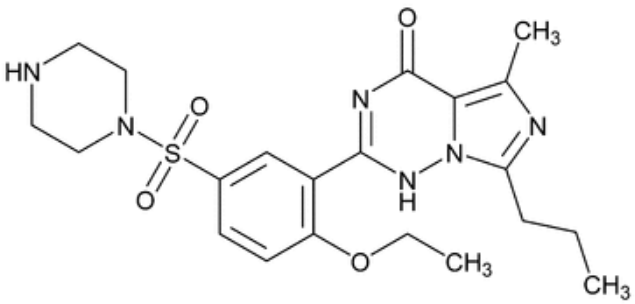
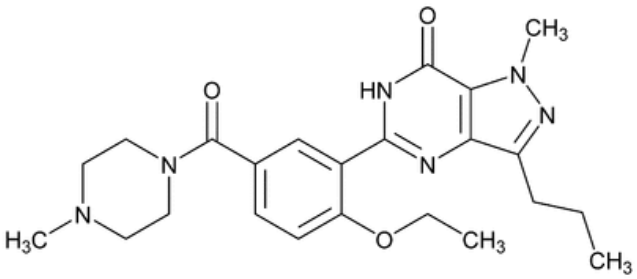
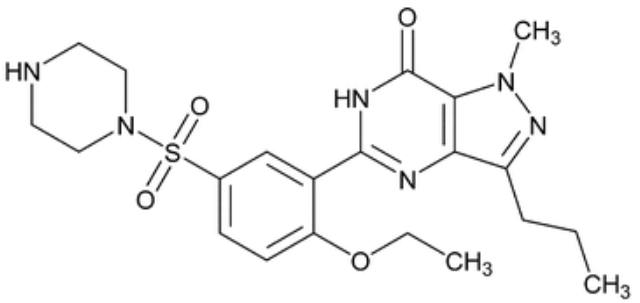


N-Desethylvardenafil

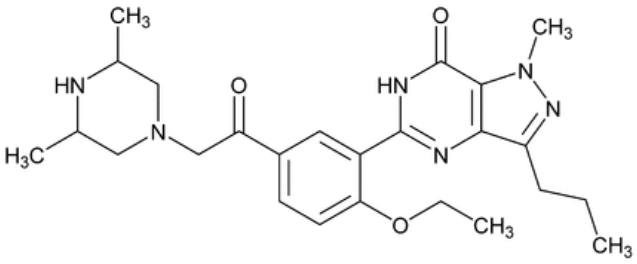


Desmethylcarbodenafil

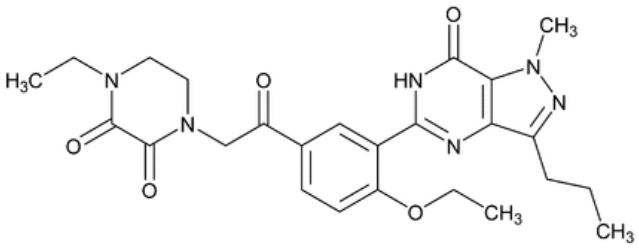


N-Desmethylsildenafil

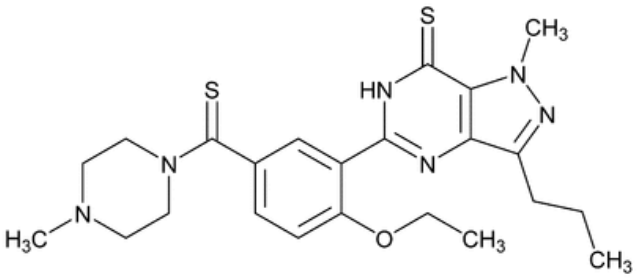
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



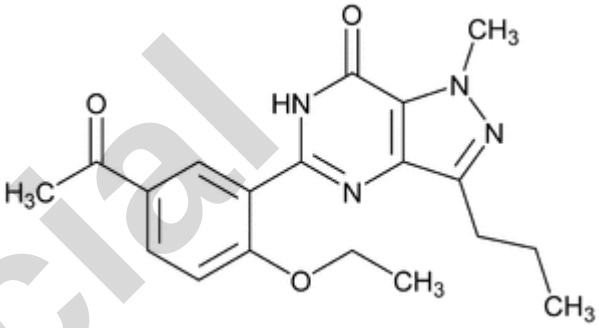
Dimethylacetildenafil



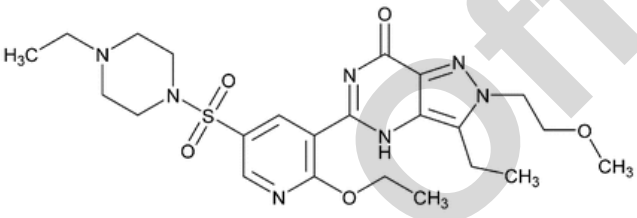
Dioxo-acetildenafil



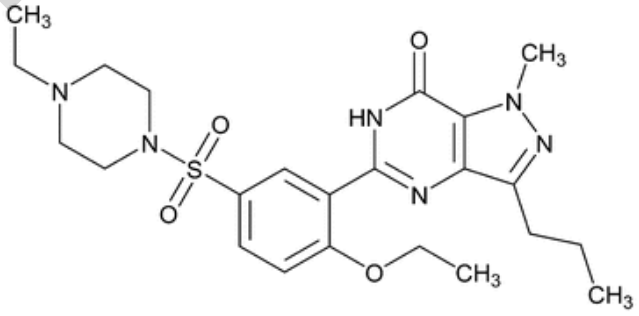
Dithio-desmethylcarbodenafil



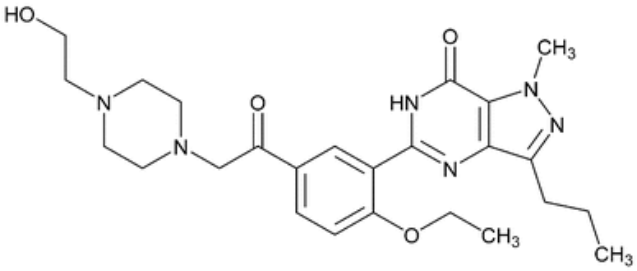
Gendenafil



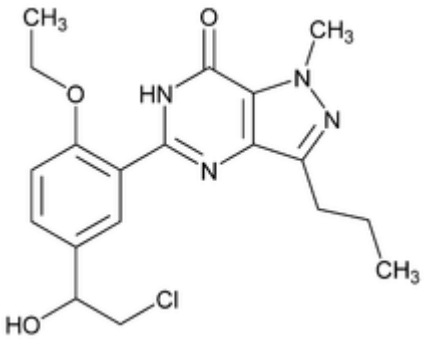
Gisadenafil



Homosildenafil

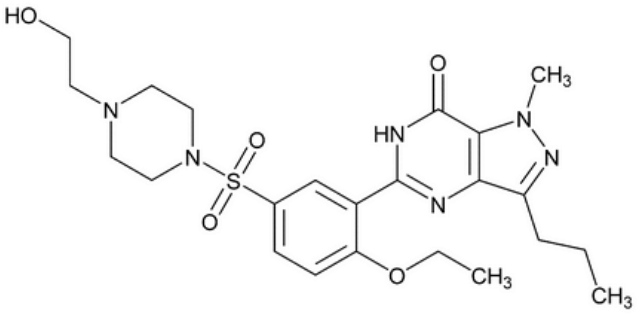


Hydroxyacetildenafil

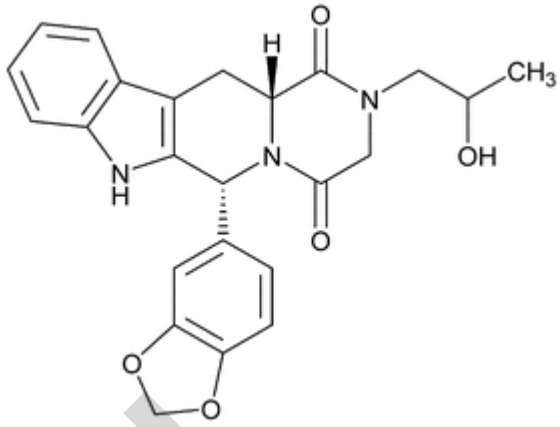


Hydroxychlorodenafil

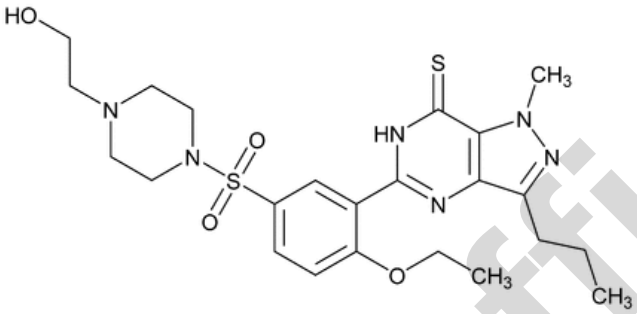
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



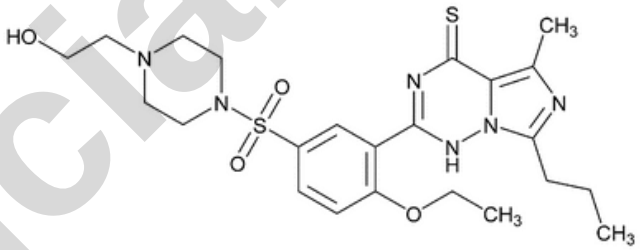
Hydroxyhomosildenafil



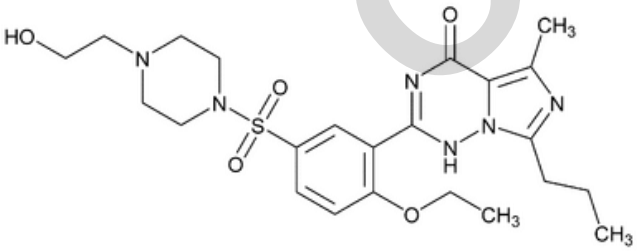
2-Hydroxypropylnortadalafil



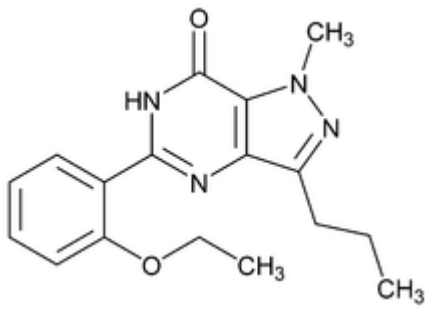
Hydroxythiohomosildenafil



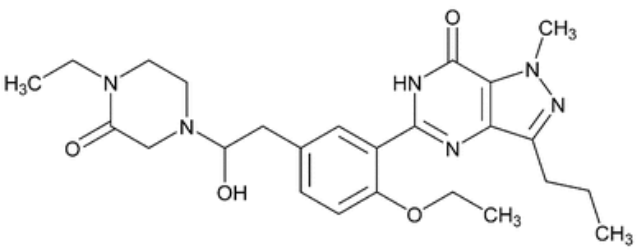
Hydroxythiovardenafil



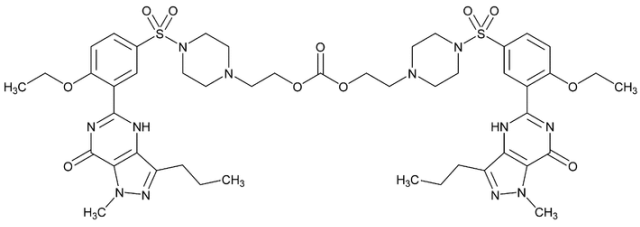
Hydroxyvardenafil



Imidazosagatriazinone

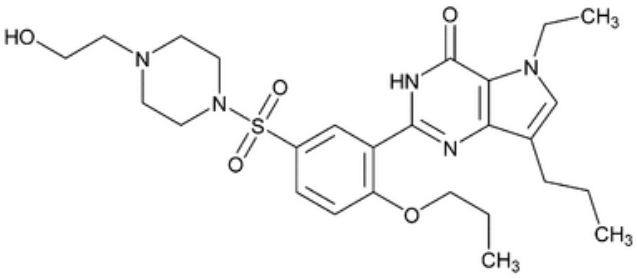


Isopiperazinonafil

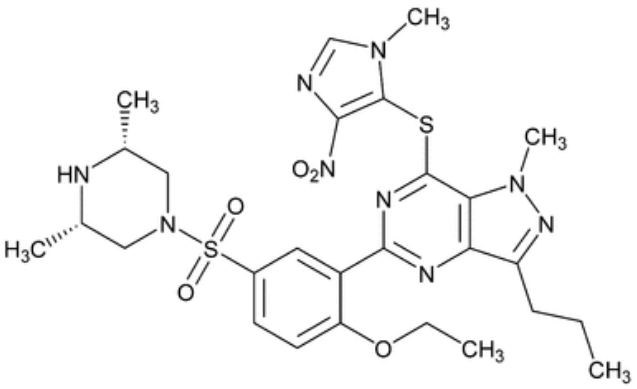


Lodenafil carbonate

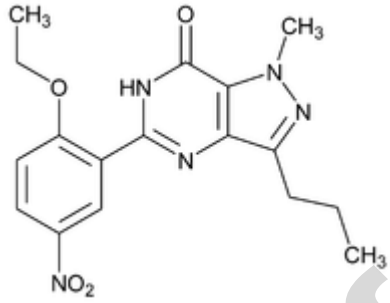
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



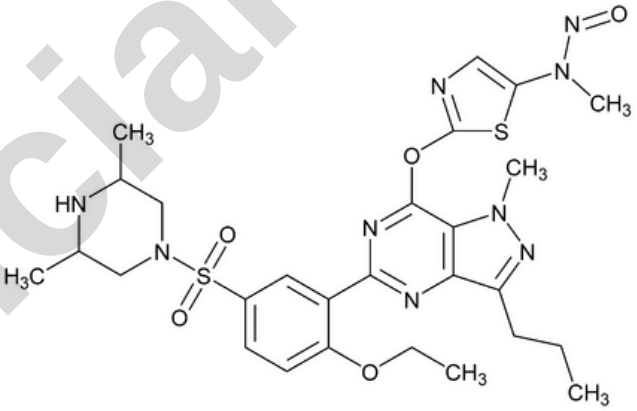
Mirodenafil



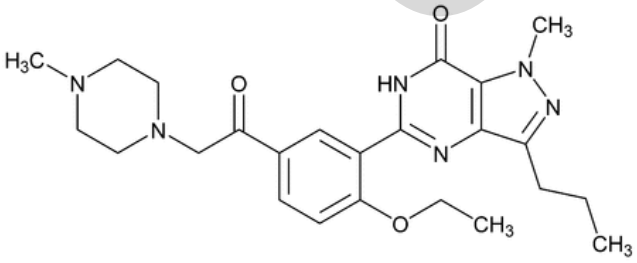
Mutaprodenafil



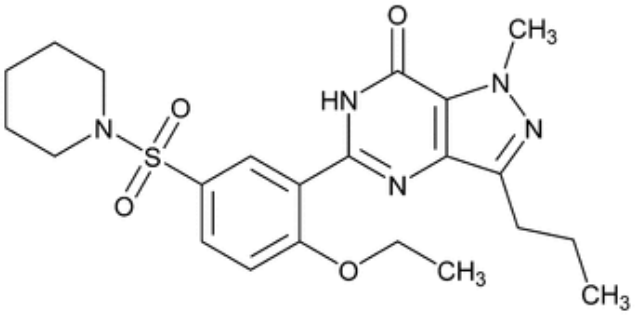
Nitrodenafil



Nitroso-prodenafil

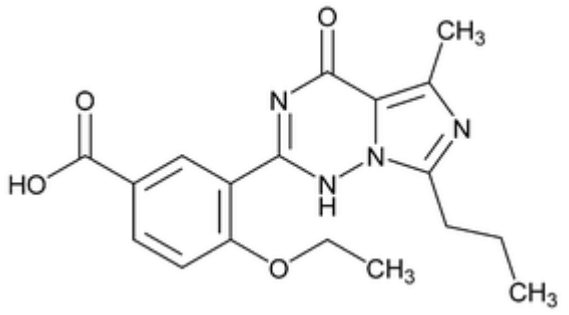


Noracetildenafil

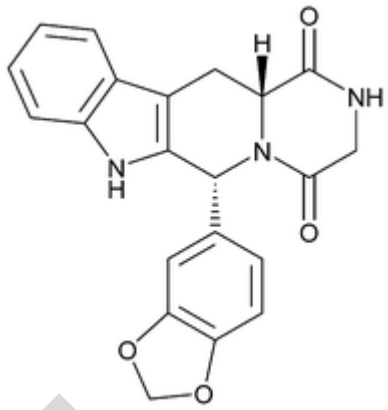


Norneosildenafil

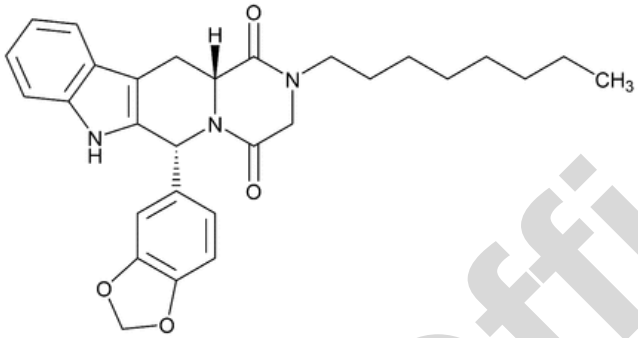
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



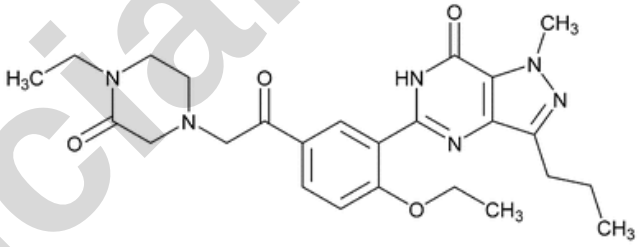
Norneovardenafil



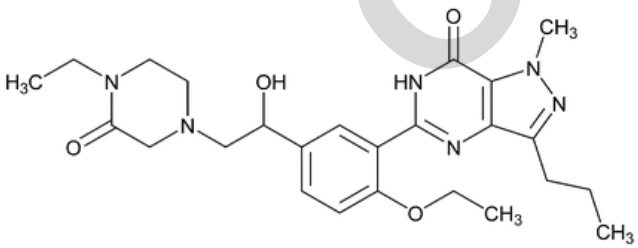
Nortadalafil



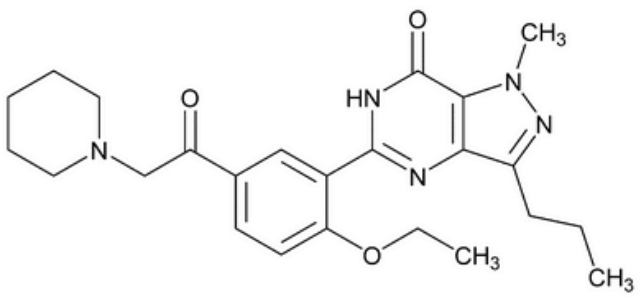
N-Octylnortadalafil



Oxohongdenafil

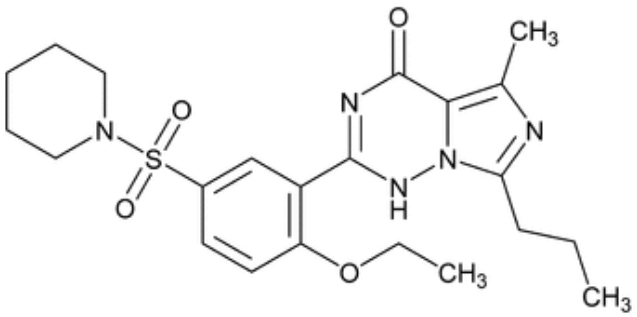


Piperazinonafil

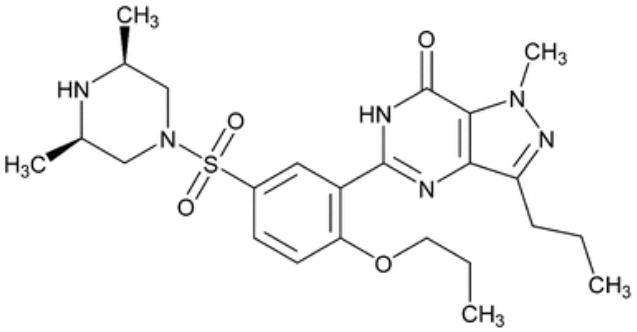


Piperidino acetildenafil

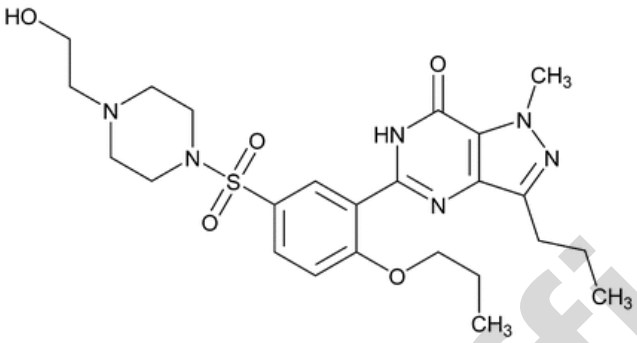
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



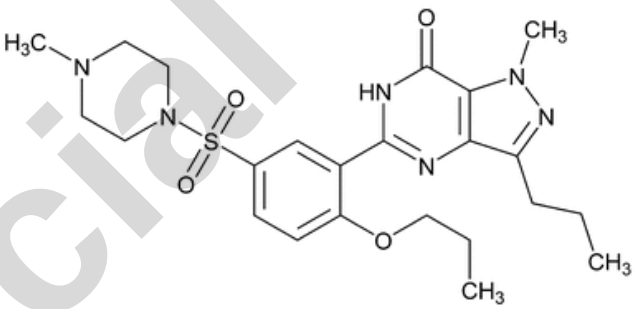
Piperidinovardenafil



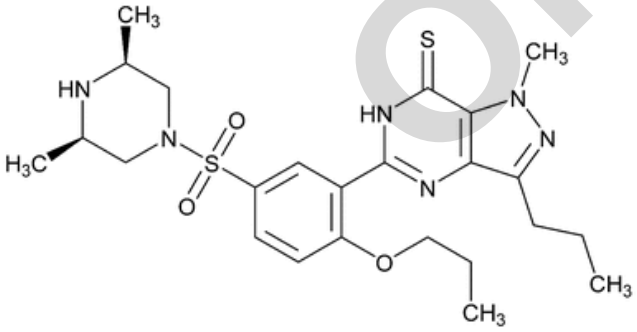
Propoxyphenyl aildenafil



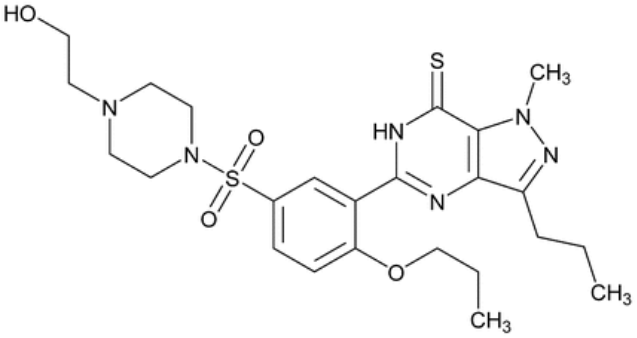
Propoxyphenyl hydroxyhomosildenafil



Propoxyphenyl sildenafil

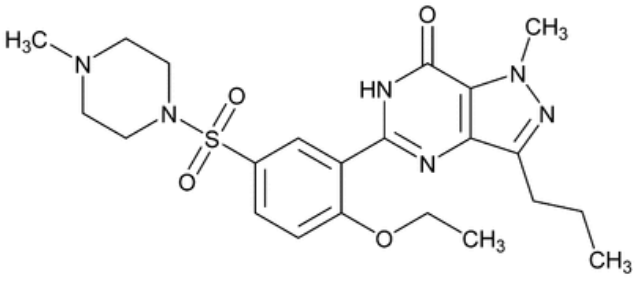


Propoxyphenyl thioaildenafil

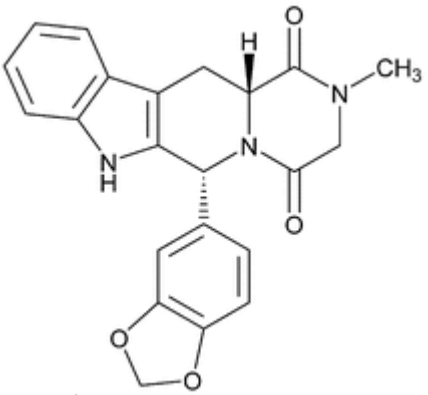


Propoxyphenyl thiohydroxyhomosildenafil

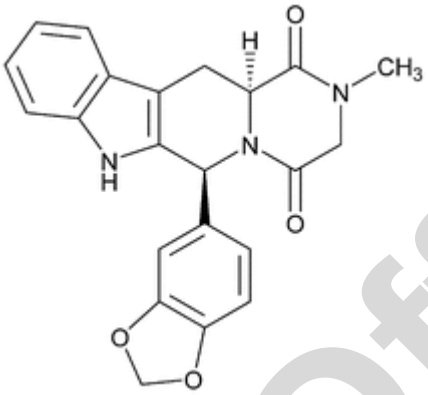
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



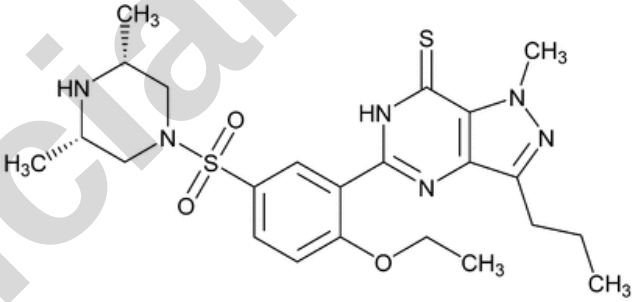
Sildenafil



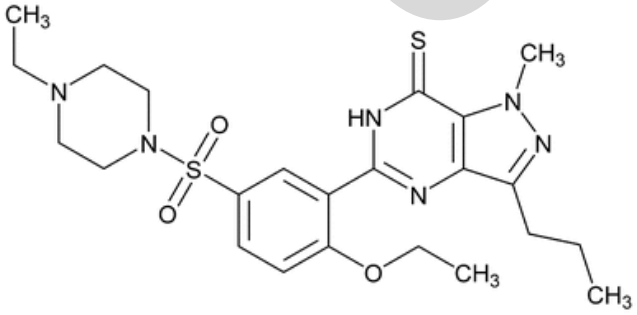
Tadalafil



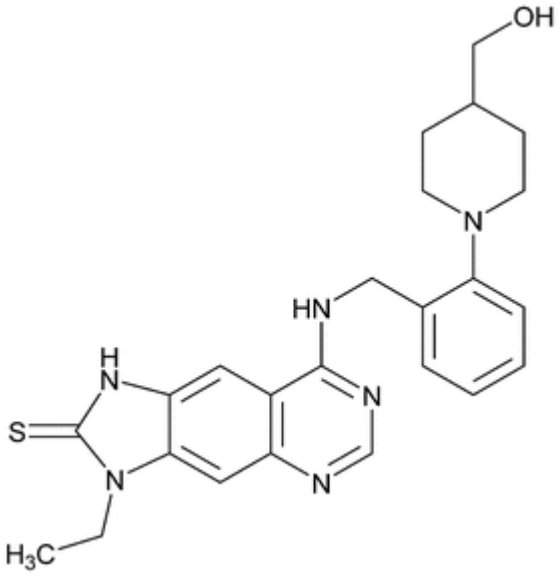
(–)-*trans*-Tadalafil



Thioaildenafil

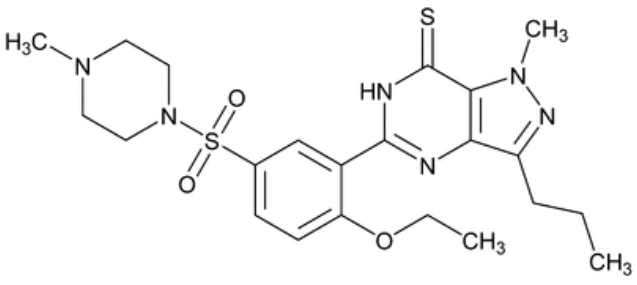


Thiohomosildenafil

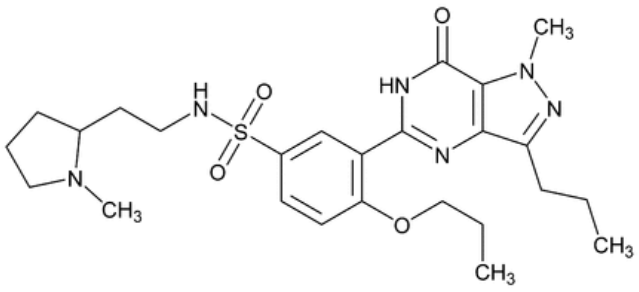


Thioquinapiperifil

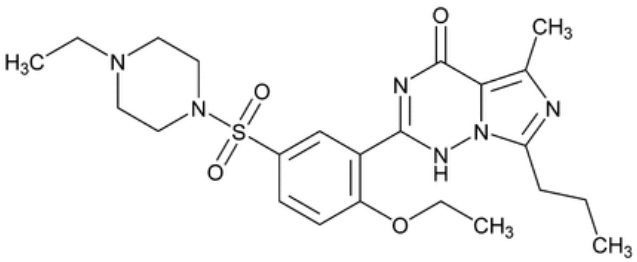
Figure 2. Chemical structures of select PDE5 inhibitors.[12] *(continued)*



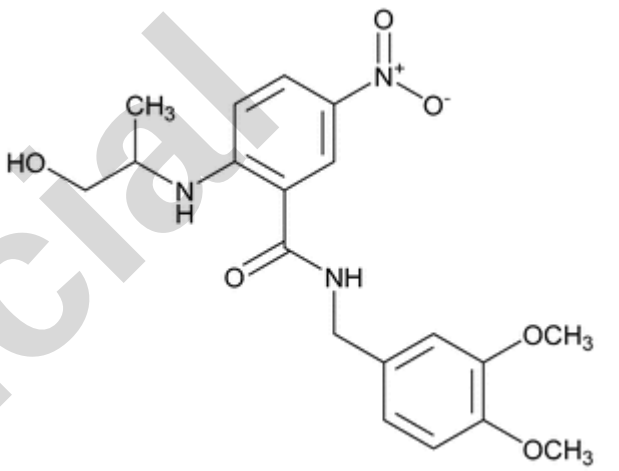
Thiosildenafil



Udenafil



Vardenafil



Xanthoanthrafil

[12] Data compiled from published sources.