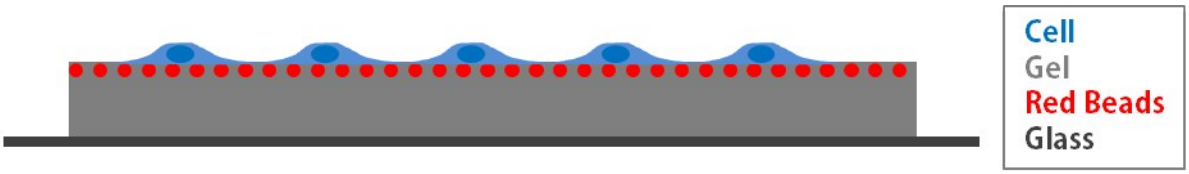
Electronic Supplementary Material (ESI) for Integrative Biology.

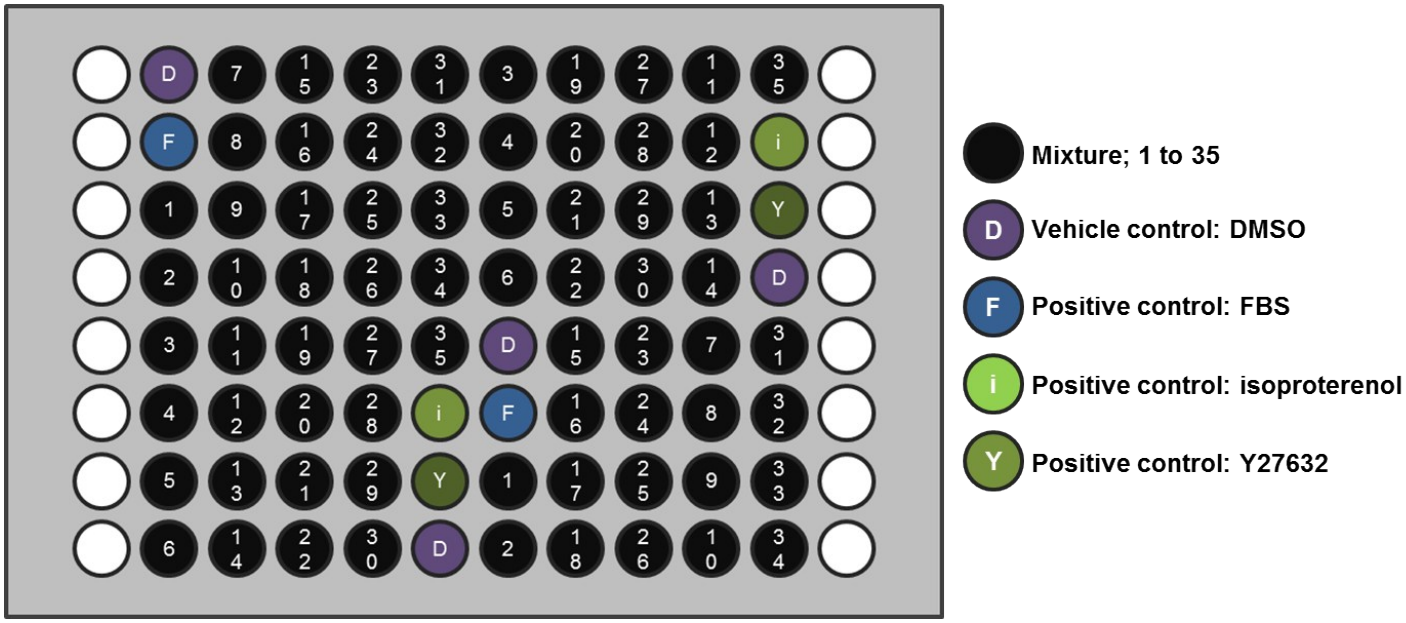
Supplementary Information

Title: High-throughput screening for modulators of cellular contractile force.

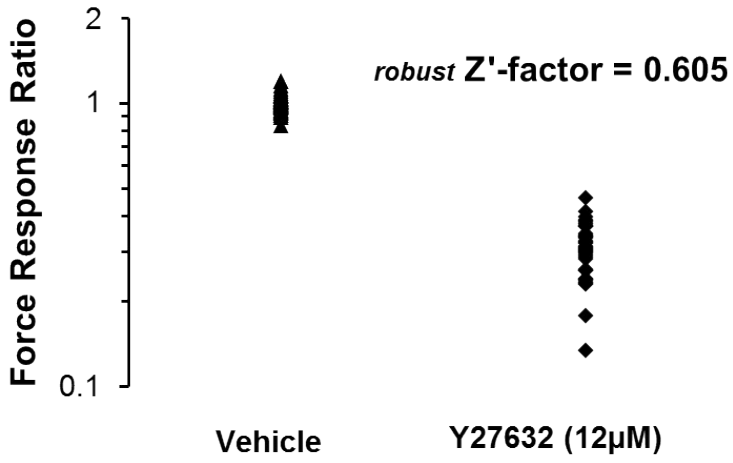
S**upplementary Figure 1: Preparation of deformable substrates in 96-well plates; an alternative method.** Acrylamide-based hydrogels (Young's modulus ≈ 2.5kPa, thickness ≈ 200μm) were miniaturized in glass bottom 96-well plates using a multi-layered approach. Both layers had the same chemical composition with the exception of 2% wt/vol of red beads (0.5μm carboxylate-modified microspheres, Invitrogen) added to the top layer. The top layer was prepared exceedingly thin to promote bead dispersion within a single horizontal plane.



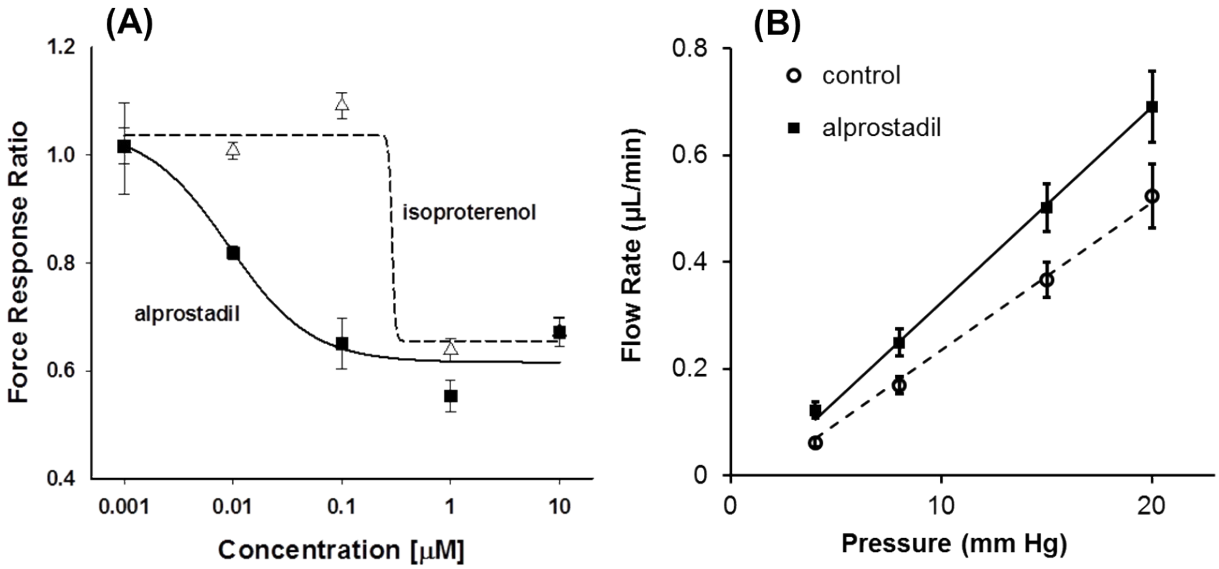
Supplementary Figure 2: Layout of drug plate. Each plate contained 35 different mixtures (1 to 35), vehicle control (0.5% DMSO), and three control drugs (1%FBS, 10μM or 25μM isoproterenol, and 10μM or 25μM Y27632).



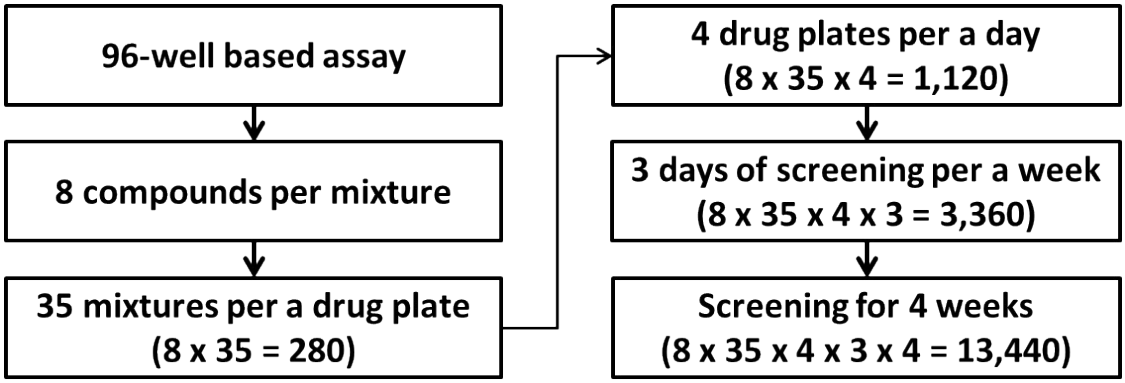
Supplementary Figure 3: Force response ratio is a high-quality screening endpoint. We assessed the sensitivity of CFS as a high-throughput screening assay using the robust Z'-factor value [1, 2]. Y27632 (rho kinase inhibitor, 12μM) was used as a positive control and water (0.01%) was used as a vehicle control. 40 positive and 40 vehicle controls were evenly distributed among 80 wells in one 96-well plate. Each symbol represents the force response ratio measured at each well. Based on these measurements, we calculated the robust Z'-factor (see Methods) and it was 0.605 which exceeds the recommended value of 0.4~0.5 for a good quality HTS screen [3, 4].



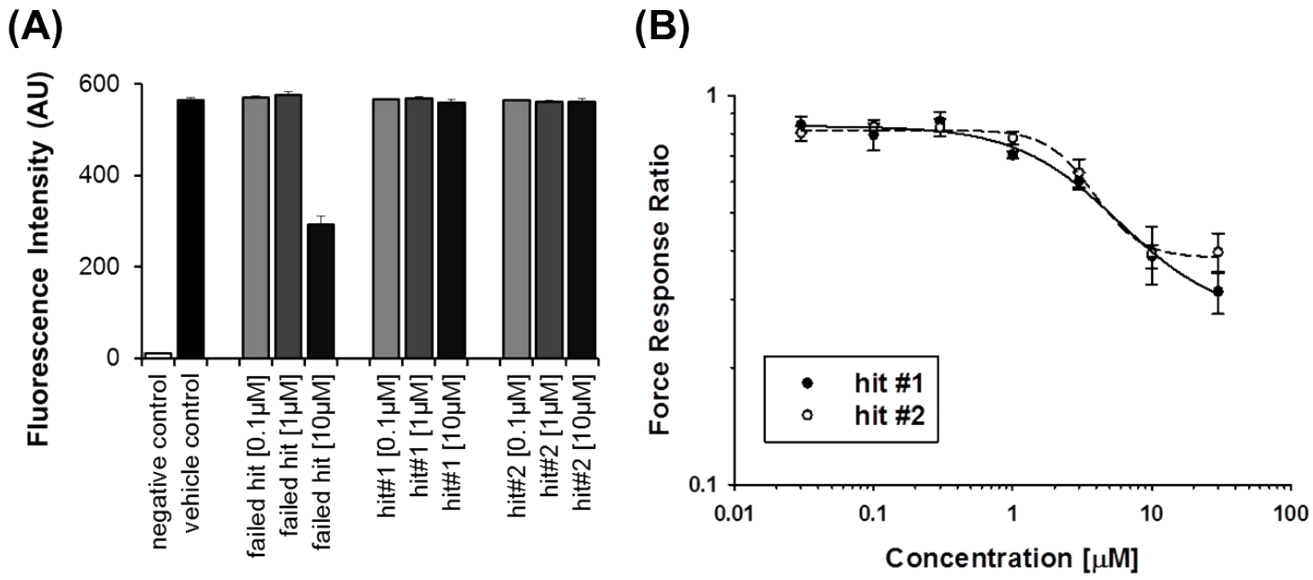
Supplementary Figure 4: Alprostadil (prostaglandin E1) relaxes SC cells and promotes outflow function in enucleated mouse eyes. (A) Alprostadil reduced SC contractile forces in a dose dependent fashion with greater potency than isoproterenol. EC50s were 290nM for isoproterenol and 9.1nM for alprostadil. (B) 1μM alprostadil increased outflow facility (inverse of flow resistance) during perfusion of enucleated mouse eyes. ($p < 2x10^{-5}$, n=11 pairs). Plotted are the average ± SEM.



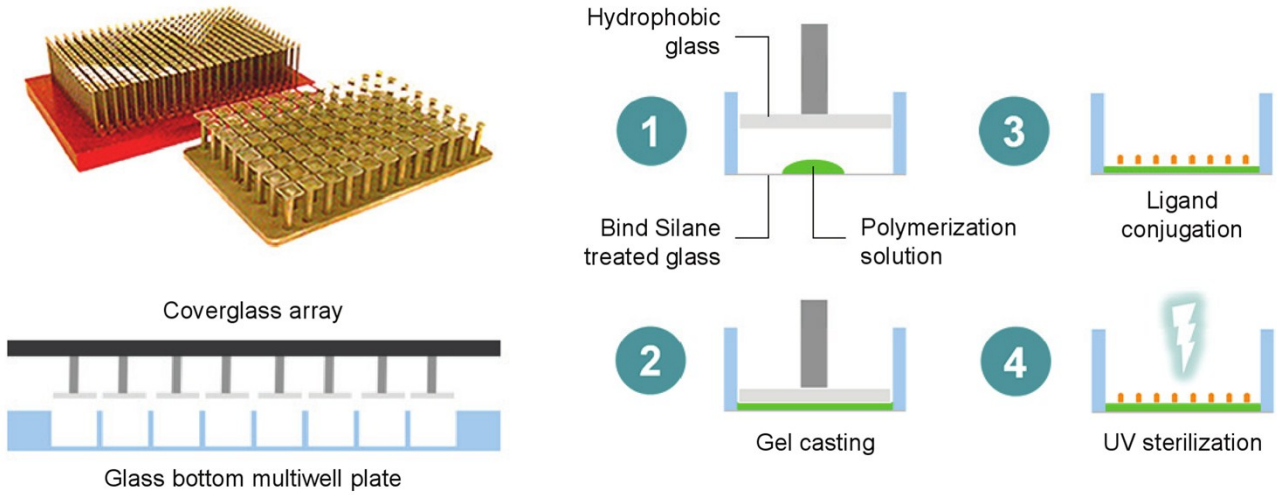
Supplementary Figure 5: High-throughput implementation of CFS. Using the Chembridge Diverset® library, we confirmed CFS throughput by screening 10,000 compounds within 4 weeks. The estimation is based on 96-well based assay plates, quadruplicate measurements, and 1hr drug incubation.



Supplementary Figure 6: Secondary validation for novel bronchodilator hits identified by CFS. (A) The 2 hits found from the Chembridge Diverset® did not affect cell viability depicted as the fluorescence intensity measured using a standard alamar Blue® assay (n=8 wells per compound per dose; 1% triton-X and 0.5% DMSO were used as negative and vehicle controls, respectively). (B) Both hit compounds reduced cellular contractile force in a dose dependent manner (n=8 wells per compound per dose). EC50s were 3.63±1.37μM for hit #1 and 3.33±0.32μM for hit #2. Plotted are the average ± SD.



Supplementary Figure 7: Schematic of gel making in a multi-well plate. This figure was reproduced from Ref [5] (Ref 30 in the main text).



Supplementary Table 1: (A) From the screening of the Prestwick library, we identified 15 individual drugs that relaxed HASM cells. (B) From the screening of the Prestwick library, we identified 17 individual drugs that relaxed SC cells.

Supplementary Table 1A:

Chemical name	Effects
Salbutamol	β2-adrenergic receptor agonist
Fenoterol hydrobromide	
Terbutaline hemisulfate	
Clenbuterol hydrochloride	
Ritodrine hydrochloride	
Metaproterenol sulfate	
Isoetharine mesylate salt	
(+)-Isoproterenol (+)-bitartrate salt	
(-)-Isoproterenol hydrochloride	
Alprostadil	Known smooth muscle relaxant
Ethaverine hydrochloride	
Kaempferol	
Chicago sky blue 6B	Inhibitor of L-glutamate uptake
Terconazole	Anti-fungal medication
Levonordefrin	Vasoconstrictor

Supplementary Table 1B:

Chemical name	Effects
Salbutamol	β2-adrenergic receptor agonist
Fenoterol hydrobromide	
Terbutaline hemisulfate	
Clenbuterol hydrochloride	
Ritodrine hydrochloride	
Metaproterenol sulfate	
Isoetharine mesylate salt	
(+)-Isoproterenol (+)-bitartrate salt	
(-)-Isoproterenol hydrochloride	
Pepstatin A	Inhibitor of aspartyl proteases, inhibits phosphorylation of ERK
Cyclosporin A	Immunosuppressant drug in organ transplantation
Clioquinol	Antifungal drug and antiprotozoal drug
Chicago sky blue 6B	Inhibitor of L-glutamate uptake
Terconazole	Anti-fungal medication
Levonordefrin	Topical nasal decongestant and vasoconstrictor in dentistry
Sanguinarine	Toxin
Alprostadil	Prostaglandin E1, known bronchodilator and vasodilator

Supplementary Table 2: Supplementary Table 2 is the expanded version of Supplementary Table 1. (A) From the screening of the Prestwick library, we chose 16 mixtures as positives and retested drugs individually. We identified 15 individual drugs that relaxed HASM cells. (B) From the screening of the Prestwick library, we chose 16 mixtures as positives and retested drugs individually. We identified 17 individual drugs that relaxed SC cells.

Supplementary Table 2A:

Chemical name	fmla structure	Mol weight	Force response	Chemical name	fmla structure	Mol weight	Force response
(-)-Isoproterenol hydrochloride	C11H18ClNO3	247.72	↓	Hymecromone	C10H8O3	176.17	_
(+)-Isoproterenol (+)-bitartrate salt	C15H23NO9	361.35	↓	Isoetharine mesylate salt	C14H25NO6S	335.42	↓
(+,-)-Synephrine	C9H13NO2	167.21	_	Kaempferol	C15H10O6	286.24	↓
2-Chloropyrazine	C4H3ClN2	114.53	_	Ketotifen fumarate	C23H23NO5S	425.51	_
3-Acetylcoumarin	C11H8O3	188.18	_	Levonordefrin	C9H13NO3	183.21	↓
6-Hydroxytropinone	C8H13NO2	155.20	_	Lidoflazine	C30H35F2N3O	491.63	_
Alprostadil	C20H34O5	354.49	↓	Lincomycin hydrochloride	C18H35ClN2O6S	443.01	_
Ascorbic acid	C6H8O6	176.13	_	Mephenesin	C10H14O3	182.22	_
Aspartic acid, N-acetyl (R,S)	C6H9NO5	175.14	_	Mephenytoin	C12H14N2O2	218.26	_
Azaperone	C19H22FN3O	327.41	_	Metaproterenol sulfate, orciprenaline sulfate	C22H36N2O10S	520.60	↓
Bambuterol hydrochloride	C18H30ClN3O5	403.91	_	Methacholine chloride	C8H18ClNO2	195.69	_
Benzathine benzylpenicillin	C48H56N6O10S2	941.14	_	Methyldopate hydrochloride	C12H18ClNO4	275.73	_
Cefaclor	C15H16ClN3O5S	385.83	_	N-Acetyl-L-leucine	C8H15NO3	173.21	_
Cefepime hydrochloride	C19H27ClN6O6S2	535.04	_	Niacin	C6H5NO2	123.11	_
Chicago sky blue 6B	C34H24N6Na4O16S4	992.82	↓	Nitrendipine	C18H20N2O6	360.37	_
Chlorpheniramine maleate	C20H23ClN2O4	390.87	_	Nitrofurantoin	C8H6N4O5	238.16	_
Clenbuterol hydrochloride	C12H19Cl3N2O	313.66	↓	Pentolinium bitartrate	C23H42N2O12	538.60	_
Clioquinol	C9H5ClINO	305.50	_	Pepstatin A	C34H63N5O9	685.91	_
Cyclopentolate hydrochloride	C17H26ClNO3	327.85	_	Ramipril	C23H32N2O5	416.52	_
Cyclosporin A	C62H111N11O12	1202.64	_	Ritodrine hydrochloride	C17H22ClNO3	323.82	↓
Dextromethorphan hydrobromide monohydrate	C18H28BrNO2	370.33	_	Ronidazole	C6H8N4O4	200.16	_
Diazoxide	C8H7ClN2O2S	230.67	_	Salbutamol	C13H21NO3	239.32	↓
Disulfiram	C10H20N2S4	296.54	_	Sanguinarine	C20H14NO4	332.34	_
Dubinidine	C15H17NO4	275.31	_	Sulfanilamide	C6H8N2O2S	172.21	_
Fenoterol hydrobromide	C17H22BrNO4	384.27	↓	Tacrine hydrochloride hydrate	C13H15ClN2	234.73	_
Flunarizine dihydrochloride	C26H28Cl2F2N2	477.43	_	Terbutaline hemisulfate	C24H40N2O10S	548.66	↓
Fluspirilen	C29H31F2N3O	475.59	_	Terconazole	C26H31Cl2N5O3	532.47	↓
Fluticasone propionate	C25H31F3O5S	500.58	_	Tomatine	C50H83NO21	1034.21	_
Gabexate mesilate	C17H27N3O7S	417.48	_	Trazodone hydrochloride	C19H23Cl2N5O	408.33	_
Guanfacine hydrochloride	C9H10Cl3N3O	282.56	_	Trimethadione	C6H9NO3	143.14	_
Hycanthone	C20H24N2O2S	356.49	_	Verteporfin	C41H44N4O9	736.83	_
Hymecromone	C10H8O3	176.17	_	Zaprinast	C13H13N5O2	271.28	_
Isoetharine mesylate salt	C14H25NO6S	335.42	↓	Zardaverine	C12H10F2N2O3	268.22	_

Supplementary Table 2B:

Chemical name	Formula	Mol weight	Force Response	Chemical name	Formula	Mol weight	Force Response
(-)-Isoproterenol hydrochloride	C11H18ClNO3	247.72	↓	Isoetharine mesylate salt	C14H25NO6S	335.42	↓
(+)-Isoproterenol (+)-bitartrate salt	C15H23NO9	361.35	↓	Kaempferol	C15H10O6	286.24	-
(+,-)-Synephrine	C9H13NO2	167.21	-	Ketotifen fumarate	C23H23NO5S	425.51	-
2-Chloropyrazine	C4H3ClN2	114.53	-	Levonordefrin	C9H13NO3	183.21	↓
3-Acetylcoumarin	C11H8O3	188.18	-	Lidoflazine	C30H35F2N3O	491.63	-
6-Hydroxytropinone	C8H13NO2	155.20	-	Lincomycin hydrochloride	C18H35ClN2O6S	443.01	-
Alprostadil	C20H34O5	354.49	↓	Mephenesin	C10H14O3	182.22	-
Aminophylline	C16H24N10O4	420.43	-	Mephenytoin	C12H14N2O2	218.26	-
Ascorbic acid	C6H8O6	176.13	-	Metaproterenol sulfate orciprenaline sulfate	C22H36N2O10S	520.60	↓
Aspartic acid, N-acetyl (R,S)	C6H9NO5	175.14	-	Methacholine chloride	C8H18ClNO2	195.69	-
Azaperone	C19H22FN3O	327.41	-	Methyldopate hydrochloride	C12H18ClNO4	275.73	-
Bambuterol hydrochloride	C18H30ClN3O5	403.91	-	N-Acetyl-L-leucine	C8H15NO3	173.21	-
Benzathine benzylpenicillin	C48H56N6O10S2	941.14	-	Niacin	C6H5NO2	123.11	-
Cefaclor	C15H16ClN3O5S	385.83	-	Nitrendipine	C18H20N2O6	360.37	-
Cefepime hydrochloride	C19H27ClN6O6S2	535.04	-	Nitrofurantoin	C8H6N4O5	238.16	-
Chicago sky blue 6B	C34H24N6Na4O16S4	992.82	↓	Pentolinium bitartrate	C23H42N2O12	538.60	-
Clenbuterol hydrochloride	C12H19Cl3N2O	313.66	↓	Pepstatin A	C34H63N5O9	685.91	↓
Clioquinol	C9H5ClINO	305.50	↓	Phentolamine hydrochloride	C17H20ClN3O	317.82	-
Cyclosporin A	C62H111N11O12	1202.64	↓	Proglumide	C18H26N2O4	334.42	-
Dextromethorphan hydrobromide monohydrate	C18H28BrNO2	370.33	-	Ramipril	C23H32N2O5	416.52	-
Ethaverine hydrochloride	C24H30ClNO4	431.96	-	Ritodrine hydrochloride	C17H22ClNO3	323.82	↓
Ethopropazine hydrochloride	C19H25ClN2S	348.94	-	Salbutamol	C13H21NO3	239.32	↓
Fenbendazole	C15H13N3O2S	299.35	-	Sanguinarine	C20H14NO4	332.34	↓
Fenoterol hydrobromide	C17H22BrNO4	384.27	↓	Sulconazole nitrate	C18H16Cl3N3O3S	460.77	-
Flunarizine dihydrochloride	C26H28Cl2F2N2	477.43	-	Sulfanilamide	C6H8N2O2S	172.21	-
Fluspirilen	C29H31F2N3O	475.59	-	Tacrine hydrochloride hydrate	C13H15ClN2	234.73	-
Fluticasone propionate	C25H31F3O5S	500.58	-	Terbutaline hemisulfate	C24H40N2O10S	548.66	↓
Gabexate mesilate	C17H27N3O7S	417.48	-	Terconazole	C26H31Cl2N5O3	532.47	↓
Guanfacine hydrochloride	C9H10Cl3N3O	282.56	-	Trazodone hydrochloride	C19H23Cl2N5O	408.33	-
Hycanthone	C20H24N2O2S	356.49	-	Trimethadione	C6H9NO3	143.14	-
Hymecromone	C10H8O3	176.17	-	Zaprinast	C13H13N5O2	271.28	-
Indapamide	C16H16ClN3O3S	365.84	-	Zardaverine	C12H10F2N2O3	268.22	-

References

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