Targeting Estrogen Receptor Subtypes in Menopausal Syndrome: Differential Effects on Vasomotor Symptoms, Mood, and Bone Health – A Systematic Review and Meta-Analysis

Appendix 1

Table S1. Full search strategy.

CLINICAL TRIALS	
PubMed (from inception to 2025)	
1 (ERalpha OR Estrogen Receptors alpha OR Receptor alpha, Estrogen OR Estradiol Receptor alpha OR alpha, Estradiol Receptor OR Receptor alpha, Estradiol OR Estrogen Receptor 1 OR ER beta OR Estrogen Receptors beta OR Receptor beta, Estrogen OR ERbetacx OR Estrogen Receptor 2 OR Estrogen Receptor* OR Receptor, Estrogen* OR Estrogen Nuclear Receptor OR Nuclear Receptor, Estrogen)	
2 (menopause OR "hot flashes" OR premenopaus* OR perimenopaus* OR postmenopaus* OR menopaus* OR "hot flush*" OR "hot flash*" OR climacteric OR "vagina* atroph*" OR "vaginal dryness" OR "Urogenital symptom" OR sweat* OR "sleep hyperhidrosis" OR insomnia OR vasomotor OR dyspareunia OR libido OR bones OR "Quality of life")	
3 (Randomized Controlled Trial OR controlled clinical trial OR random allocation OR double-blind OR singled-blind OR Placebo OR Randomly OR randomized OR clinical trial* OR RCT OR Random*)	
Strategy of search	**Results**
1 AND 2 AND 3	**4994**
Web of Science (from inception to 2025)	
1 TS= (ERalpha OR Estrogen Receptors alpha OR Receptor alpha, Estrogen OR Estradiol Receptor alpha OR alpha, Estradiol Receptor OR Receptor alpha, Estradiol OR Estrogen Receptor 1 OR ERbeta OR Estrogen Receptors beta OR Receptor beta, Estrogen OR ERbetacx OR Estrogen Receptor 2 OR Estrogen Receptor* OR Receptor, Estrogen* OR Estrogen Nuclear Receptor OR Nuclear Receptor, Estrogen)	
2 TS= (menopause OR "hot flashes" OR premenopaus* OR perimenopaus* OR postmenopaus* OR menopaus* OR "hot flush*" OR "hot flash*" OR climacteric OR "vagina* atroph*" OR "vaginal dryness" OR "Urogenital symptom" OR sweat* OR "sleep hyperhidrosis" OR insomnia OR vasomotor OR dyspareunia OR libido OR bones OR "Quality of life")	
3 TS= (Randomized Controlled Trial OR controlled clinical trial OR random allocation OR double-blind OR singled-blind OR Placebo OR Randomly OR randomized OR clinical trial* OR RCT OR Random*)	
Strategy of search	**Results**
1 AND 2 AND 3	**6136**
Embase (from inception to 2025)	
1 (ERalpha OR Estrogen Receptors alpha OR Receptor alpha, Estrogen OR	

Estradiol Receptor alpha OR alpha, Estradiol Receptor OR Receptor alpha, Estradiol OR Estrogen Receptor 1 OR ERbeta OR Estrogen Receptors beta OR Receptor beta, Estrogen OR ERbetacx OR Estrogen Receptor 2 OR Estrogen Receptor* OR Receptor, Estrogen* OR Estrogen Nuclear Receptor OR Nuclear Receptor, Estrogen)
2 (menopause OR "hot flashes" OR premenopaus* OR perimenopaus* OR postmenopaus* OR menopaus* OR "hot flush*" OR "hot flash*" OR climacteric OR "vagina* atroph*" OR "vaginal dryness" OR "Urogenital symptom" OR sweat* OR "sleep hyperhidrosis" OR insomnia OR vasomotor OR dyspareunia OR libido OR bones OR "Quality of life")
3 (Randomized Controlled Trial OR controlled clinical trial OR random allocation OR double-blind OR singled-blind OR Placebo OR Randomly OR randomized OR clinical trial* OR RCT OR Random*)

Strategy of search	**Results**
1 AND 2 AND 3	**3021**
Cochrane (from inception to 2025)	
1 (ERalpha OR Estrogen Receptors alpha OR Receptor alpha, Estrogen OR Estradiol Receptor alpha OR alpha, Estradiol Receptor OR Receptor alpha, Estradiol OR Estrogen Receptor 1 OR ERbeta OR Estrogen Receptors beta OR Receptor beta, Estrogen OR ERbetacx OR Estrogen Receptor 2 OR Estrogen Receptor* OR Receptor, Estrogen* OR Estrogen Nuclear Receptor OR Nuclear Receptor, Estrogen)	
2 (menopause OR "hot flashes" OR premenopaus* OR perimenopaus* OR postmenopaus* OR menopaus* OR "hot flush*" OR "hot flash*" OR climacteric OR "vagina* atroph*" OR "vaginal dryness" OR "Urogenital symptom" OR sweat* OR "sleep hyperhidrosis" OR insomnia OR vasomotor OR dyspareunia OR libido OR bones OR "Quality of life")	
3 (Randomized Controlled Trial OR controlled clinical trial OR random allocation OR double-blind OR singled-blind OR Placebo OR Randomly OR randomized OR clinical trial* OR RCT OR Random*)	
Strategy of search	**Results**
1 AND 2 AND 3	**2972**

Table S2. Sensitivity Analysis of menopausal symptom scores (leave-one-out method)

Studies excluded	SMD	95% CI		I^2	*P* Value
		Lower	Upper		
None (Overall Pooled)	-1.95	-3.18	-0.72	98%	＜0.01
Hasper I et al. (2009)	-1.57	-2.80	-0.35	97%	＜0.05
Heger M et al. (2006)	-1.81	-3.17	-0.44	98%	＜0.01
Atkinson C et al. (2004)	-2.34	-3.62	-1.05	97%	＜0.001
Kaszkin-Bettag M et al. (2009)	-1.84	-3.24	-0.45	98%	＜0.05
Gaspard U et al. (2023)	-2.31	-3.75	-0.86	98%	＜0.01
Thiemann E et al. (2017)	-1.82	-3.19	-0.44	98%	＜0.01

Abbreviations: CI, Confidence Interval; SMD, Standardized Mean Difference. Note: Results represent the pooled effect size and heterogeneity calculated after excluding the indicated study. *P* value indicates the significance of the pooled SMD.

Table S3. ***Sensitivity Analysis of Hot Flashes (leave-one-out method)***

Studies excluded	SMD	95% CI		I^2	*P* Value
		Lower	Upper		
None (Overall Pooled)	-1.21	-1.88	-0.54	91%	＜0.001
Hasper I et al. (2009)	-1.00	-1.67	-0.32	90%	＜0.01
Heger M et al. (2006)	-1.04	-1.75	-0.33	90%	＜0.01
Schneider S et al. (2019)	-1.40	-2.11	-0.70	91%	＜0.001
Kaszkin-Bettag M et al. (2009)	-1.15	-1.98	-0.32	93%	＜0.01
Guojun Cheng et al. (2007)	-1.31	-2.08	-0.53	92%	＜0.01
Gaspard U et al. (2020)	-1.36	-2.08	-0.64	90%	＜0.001

Abbreviations: CI, Confidence Interval; SMD, Standardized Mean Difference.Note: Results represent the pooled effect size and heterogeneity calculated after excluding the indicated study. *P* value indicates the significance of the pooled SMD.

Table S4. Sensitivity Analysis of depression (leave-one-out method)

Studies excluded	SMD	95% CI		I^2	*P* Value
		Lower	Upper		
None (Overall Pooled)	-1.13	-2.01	-0.26	93%	0.01
Hasper I et al. (2009)	-0.81	-1.70	0.07	91%	0.07
Heger M et al. (2006)	-0.89	-1.89	0.10	92%	0.08
Hirose et al. (2016)	-1.23	-2.25	-0.21	93%	0.02
Kaszkin-Bettag M et al. (2009)	-1.07	-2.25	0.10	94%	0.07
Schmidt P J et al. (2021)	-1.22	-2.21	-0.23	94%	0.02
Schmidt P J et al. (2021) [Rimostil]	-1.53	-2.30	-0.76	90%	＜0.001

Abbreviations: CI, Confidence Interval; SMD, Standardized Mean Difference. Note: Results represent the pooled effect size and heterogeneity calculated after excluding the indicated study. *P* value indicates the significance of the pooled SMD.

Table S5. GRADE summary of findings for estrogen receptor-targeted interventions versus placebo for menopausal symptom relief

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	ER-targeted interventions	Placebo	Relative (95% CI)	Absolute (95% CI)		
Menopause Rating Scale (assessed with: Menopause Rating Scale or other validated menopausal symptom scales)												
6	randomised trials	serious[a]	very serious[b]	not serious	not serious	none	311	335	-	SMD **1.95 SD lower** (3.18 lower to 0.72 lower)	⨁◯◯◯ Very low[a,b]	CRITICAL
Anxiety												
5	randomised trials	serious[a]	very serious[c]	not serious	not serious	none	230	225	-	SMD **1.35 SD lower** (2.26 lower to 0.45 lower)	⨁◯◯◯ Very low[a,c]	CRITICAL
Hot flashes												
6	randomised trials	serious[a]	very serious[d]	not serious	not serious	none	245	255	-	SMD **1.21 SD lower** (1.88 lower to 0.54 lower)	⨁◯◯◯ Very low[a,d]	CRITICAL
Depression												
5	randomised trials	serious[a]	very serious[e]	not serious	not serious	none	205	199	-	SMD **1.13 SD lower** (2.01 lower to 0.26 lower)	⨁◯◯◯ Very low[a,e]	CRITICAL

CI: confidence interval; **SMD:** standardised mean difference

Explanations

a. Downgraded one level because several contributing studies had unclear reporting of random sequence generation, allocation concealment, and/or blinding of outcome assessment.
b. Downgraded two levels for very serious inconsistency because heterogeneity was very high ($I^2 = 98\%$) and the 95% prediction interval crossed the null value (-6.15 to 2.26).
c. Downgraded two levels for very serious inconsistency because heterogeneity was very high ($I^2 = 93\%$) and the 95% prediction interval crossed the null value (-5.28 to 2.62).
d. Downgraded two levels for very serious inconsistency because heterogeneity was very high ($I^2 = 91\%$) and the 95% prediction interval crossed the null value (-3.53 to 1.11).
e. Downgraded two levels for very serious inconsistency because heterogeneity was very high ($I^2 = 93\%$) and the 95% prediction interval crossed the null value (-4.70 to 2.47).

Table S6. Pharmacological basis for estrogen receptor subtype classification of included interventions.

Intervention	Assigned ER group	Detailed pharmacological rationale	Compound type	Relevant tissue/outcome context	Key supporting references
Estetrol (E4)	ERα-predominant	In vitro and in vivo studies demonstrate preferential binding and functional activation of ERα relative to ERβ. Mechanistic studies further indicate predominant nuclear ERα signaling with limited membrane ERα activation and relatively weak ERβ-mediated activity.	Native estrogen (NEST)	Bone, vasomotor, genitourinary	1: A multicenter, randomized study to select the minimum effective dose of estetrol (E4) in postmenopausal women (E4Relief): part 1. Vasomotor symptoms and overall safety； 2:The uterine and vascular actions of estetrol delineate a distinctive profile of estrogen receptor alpha modulation, uncoupling nuclear and membrane activation； 3: Membrane and nuclear estrogen receptor alpha actions: from tissue specificity to medical implications.4: Predominant role of nuclear versus membrane estrogen receptor alpha in arterial protection: implications for estrogen receptor alpha modulation in cardiovascular prevention/safety.
ERr 731®	ERβ-preferential	In vitro and in vivo studies demonstrate preferential binding and functional activation of ERβ over ERα. Aglycones and stilbene derivatives drive ERβ-mediated effects;limited ERα activation reported	Phytoestrogenic extract	Vasomotor, CNS/psychological symptoms, genitourinary	1: Efficacy evaluation of standardized Rheum rhaponticum root extract (ERr 731®) on symptoms of menopause: A systematic review and meta-analysis study； 2: Activation of estrogen receptor-β by a special extract of Rheum rhaponticum (ERr 731®), its aglycones and structurally related compounds.； 3: Activation of estrogen receptor-ß by a special extract of Rheum rhaponticum (ERr 731®), its aglycones and structurally related compounds.
Red clover-derived isoflavones	ERβ-preferential / mixed ER modulator	Isoflavones including genistein, daidzein, biochanin A, and formononetin demonstrate mixed ER activity with relatively greater ERβ binding affinity and transcriptional activation reported in mechanistic studies.	Phytoestrogenic extract	Vasomotor symptoms, breast tissue safety, menopausal symptoms	1: Mechanisms enforcing the estrogen receptor β selectivity of botanical estrogens; 2: Evaluation of the Estrogenic Effects of Legume Extracts Containing Phytoestrogens; 3: Phytoestrogens derived from red clover: An alternative to estrogen replacement therapy? 4:Red clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial
Isoflavones	ERβ-preferential / mixed ER modulator	Isoflavones including genistein, daidzein, and glycitein exhibit mixed ER activity with relatively greater ERβ binding affinity and transcriptional activity reported in mechanistic studies. Clinical and experimental evidence suggests preferential modulation of ERβ-related pathways with limited	Phytoestrogenic extract	Vasomotor symptoms, menopausal symptoms, breast/endometrial safety	1: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta; 2: Mechanisms enforcing the estrogen receptor β selectivity of botanical estrogens; 3: Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors β and α; 4: Isoflavone treatment for acute menopausal symptoms

		proliferative ERα stimulation in breast and endometrial tissues.			
Isoflavone aglycone	ERβ-preferential / mixed ER modulator	Isoflavone aglycones enriched in genistein and daidzein demonstrate preferential ERβ binding affinity and ERβ-associated transcriptional activation in mechanistic studies. Enhanced bioavailability of aglycone forms may facilitate CNS-related ERβ signaling implicated in mood and psychological symptom modulation with limited proliferative ERα stimulation.	Phytoestrogenic extract	Psychological symptoms, CNS/neuropsychological symptoms, menopausal symptoms	1: Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors β and α; 2: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta; 3: Mechanisms enforcing the estrogen receptor β selectivity of botanical estrogens; 4: Low-dose isoflavone aglycone alleviates psychological symptoms of menopause in Japanese women: a randomized, double-blind, placebo-controlled study
Raloxifene hydrochloride	ERα-predominant / tissue-selective SERM	Raloxifene is a selective estrogen receptor modulator (SERM) exhibiting tissue-specific agonistic and antagonistic estrogen receptor activity. Its skeletal protective effects are primarily associated with ERα-mediated signaling, supported by the observed association between ESR1 polymorphisms and therapeutic response, whereas no significant association with ESR2 polymorphisms was identified. Raloxifene acts as an estrogen agonist in bone while exerting antagonistic effects in breast and uterine tissues.	Selective estrogen receptor modulator (SERM)	Bone mineral density, bone turnover, osteoporosis, breast/endometrial safety	1: Raloxifene: mechanism of action, effects on bone tissue, and applicability in clinical traumatology practice; 2: Association of bone metabolism related genes polymorphisms with the effect of raloxifene hydrochloride on bone mineral density and bone turnover markers in postmenopausal women with osteoporosis
Rimostil	ERβ-preferential / mixed ER modulator	Rimostil is a red clover-derived phytoestrogen preparation containing isoflavones including genistein, which has relatively greater affinity for ERβ. The intervention was investigated as a putative ERβ-targeting therapy for perimenopausal depression	Phytoestrogenic extract	Perimenopausal depression, mood symptoms, menopausal neuropsychological symptoms	1: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta; 2: Mechanisms enforcing the estrogen receptor β selectivity of botanical estrogens; 3: The short-term effects of estradiol, raloxifene, and a phytoestrogen in women with perimenopausal depression
ERβ-targeted phytoSERM formulation	ERβ-selective / ERβ-targeted phytoSERM	The phytoSERM formulation was specifically designed to preferentially target ERβ-mediated signaling pathways using a combination of phytoestrogenic	PhytoSERM formulation	Menopausal symptoms, neuroprotection, CNS-related menopausal	1: Mechanisms enforcing the estrogen receptor β selectivity of botanical estrogens; 2: Safety and feasibility of estrogen receptor-β targeted phytoSERM formulation for menopausal symptoms: phase 1b/2a randomized clinical trial

		compounds with enhanced ERβ selectivity and reduced ERα-associated proliferative activity. The intervention aims to retain neuroprotective and menopausal symptom benefits while minimizing stimulation of breast and endometrial tissues.		manifestations, breast/endometrial safety	

Table S7. Summary of random-effects meta-analyses and univariable meta-regression results

Outcome	k	95% Prediction Interval	Moderator	β (95% CI)	*P*-value	R^2 (%)
Menopausal symptom score	6	-6.15 to 2.26	Duration	0.064 (-0.048 to 0.177)	0.188	23.7
	5	—	Mean age	0.508 (0.200 to 0.816)	0.014	89
Anxiety	6	-5.28 to 2.62	Duration	-0.640 (-1.088 to -0.192)	0.017	79.5
	6	—	Mean age	1.904 (0.663 to 3.145)	0.013	84
Hot flashes	6	-3.53 to 1.11	Duration	-0.128 (-0.687 to 0.431)	0.561	0
	6	—	Mean age	0.202 (0.017 to 0.386)	0.039	70.3
Depression	6	-4.70 to 2.47	Duration	-0.492 (-0.973 to -0.011)	0.047	64.7
	6	—	Mean age	1.150 (-0.848 to 3.148)	0.185	24.8

β represents the change in SMD per 1-week increase in duration or per 1-year increase in mean age.Prediction intervals are reported for the overall random-effects meta-analysis of each outcome (shown once per outcome).k indicates the number of comparisons included in each analysis.