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Teaser The soft drug approach is an effective strategy to define the action of topical dermatological drugs in the skin and obtain safer drugs.

Soft drugs for dermatological applications: recent trends

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A soft drug (SD) displays a metabolically labile spot and, after having exerted its activity in the site of action, undergoes a fast metabolism, leading to inactive metabolites. The SD approach has recently found widespread application in the dermatological field because it provides a means of localising the therapeutic effect in skin, while minimising systemic exposure. The literature is rapidly growing of successful examples of compounds targeting sphingosine-1-phosphate receptor 1 (S1PR1), transient receptor potential vanilloid 1 (TRPV1), Janus kinase (JAK), caspase 1, and histone deacetylase (HDAC), for the treatment of skin inflammatory, autoimmune, and oncological diseases. As a demonstration of the potential of this strategy, the SD approach recently led to the approval of crisaborole, a soft phosphodiesterase 4 (PDE4) inhibitor, for atopic dermatitis, while other agents are in clinical development.

Introduction

Opposite to prodrugs and at times improperly named 'antedrugs', SDs are active compounds that display a metabolically labile designed-in spot, named the 'soft spot'. This allows the drug to act in a particular compartment, but confers significant lability in other sites, thereby giving it organ specificity. Usually, these drugs are topically administered (e.g., eye, skin, intestines, nasal mucosa, or lung) and are rapidly inactivated when leaving the site of action, leading to no, or minute, systemic exposure and, therefore, increasing the therapeutic index. Over the years, the term 'SD' has been extended to compounds that are specifically designed to be vulnerable to rapid biotransformation into inactive metabolites. The anaesthesiology field, where a rapid offset is often desired, is particularly rich in short-acting drugs for systemic use [1]. There are no clear boundaries between a SD properly so called, that is a topical drug endowed with a controlled metabolic fate, and a systemic drug that is merely characterised by a short half-life; however, the former meaning is the one we mainly refer to in this review. In this narrow sense, SDs require the achievement of an adequate balance between the stability necessary to exert the desired biological activity in the site of action and the lability required to quench the compound in other sites, where undesired toxicities might occur. As discussed here, the key to achieving this is

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the exploitation of the existing differences in terms of the expression and activity of metabolizing enzymes between the site of action and the other districts.

When the SD principle was first introduced during the late 1970s, accidental SDs were already in use [2], including: etomidate (**1**, Fig. 1), a short-acting hypnotic agent; succinylcholine (**2**, Fig. 1); ester-type local anaesthetics, such as procaine (**3**, Fig. 1); and methylphenidate (**4**, Fig. 1), an amphetamine-related drug used for the treatment of attention-deficit-hyperactivity disorder. Soon after its formal introduction, the SD concept found application in the discovery of soft antimicrobial [3], antitumor [4], and anticholinergic [5] compounds, as described in the seminal papers published by Nicholas Bodor in 1980. Two years later, Henry Joung Lee coined the word 'antedrug', with the same concept as SDs, with reference to anti-inflammatory steroids devoid of pituitary-adrenal suppressive effect [6]. However, the expression of antedrugs has received little attention over the years and has been almost limited to the corticosteroid field.

The initial successful exploitation of the SD concept by Bodor and Lee boosted the rational discovery of SDs that brought to the market other soft active principles, such as remifentanil (**5**, Fig. 1), a short-acting opioid, and esmolol (**6**, Fig. 1) and landiolol (**7**, Fig. 1), two ultrashort-acting beta-blockers, providing clear proof of the validity of the concept. A recent example is revefenacin (**8**, Fig. 1), a lung-selective muscarinic antagonist approved in 2018 in the USA for chronic obstructive pulmonary disease (COPD) [7].

This topic and related examples of SDs in different therapeutic areas have been extensively reviewed elsewhere [8–12]; therefore, we invite the reader to refer to these references for the general principles and properties of SDs, together with the different SD design methods (inactive metabolite-based SD, soft analogs, active metabolite-based SD, activated SD, and pro-SD). Here, we mainly focus on the application of the SD concept in the design and development of active principles for topical dermatological use, a setting to which the SD principle is ideally suited.

Softness criteria for topical dermatological drugs

Most skin diseases that are potentially targeted by dermatological drugs are not severe, and it follows that, for therapies targeting skin, an ideal risk–benefit ratio would want not to consider systemic adverse effects. Topical administration is not always enough to guarantee the safety of the product, because the active principle might be absorbed through the skin, and, because of vascularization, reach the systemic circulation, with systemic adverse effects. Moreover, if the disease affects a large body surface area, the dose becomes higher and the risk of systemic effects more significant.

Therefore, developing a SD can be a strategy to increase the therapeutic index. A topical dermatological drug is considered soft when it is stable or slowly degraded in the skin, while being promptly metabolised in other sites (mainly plasma and liver), therefore giving low systemic exposure. SDs for topical application should fulfil the following softness criteria: (i) low skin clearance to guarantee the therapeutic effect; (ii) high systemic clearance to avoid systemic adverse effects; (iii) avoidance of metabolism mediated by saturable pathways (e.g., oxidation) in favour of a metabolism carried out by prompt enzymes that have a different expression depending on organ or tissue (e.g., hydrolysis); and (iv) generation of inactive metabolites.

To develop a topical dermatological SD with an ideal profile for clinical development, these softness criteria should be taken into account at an early stage of drug research and development (R&D) and experimentally investigated. This is not always the case and there is a rich body of literature that claims the softness of a topical dermatological drug without having confirmed the above requirements.

Considerations of metabolism in SD design

Besides being a physical barrier, the skin is considered as an extrahepatic metabolizing organ. Although catalytic activities of metabolising enzymes are observed at relatively low levels per square centimetre of skin compared with other organs, skin-mediated metabolism becomes significant when considering the

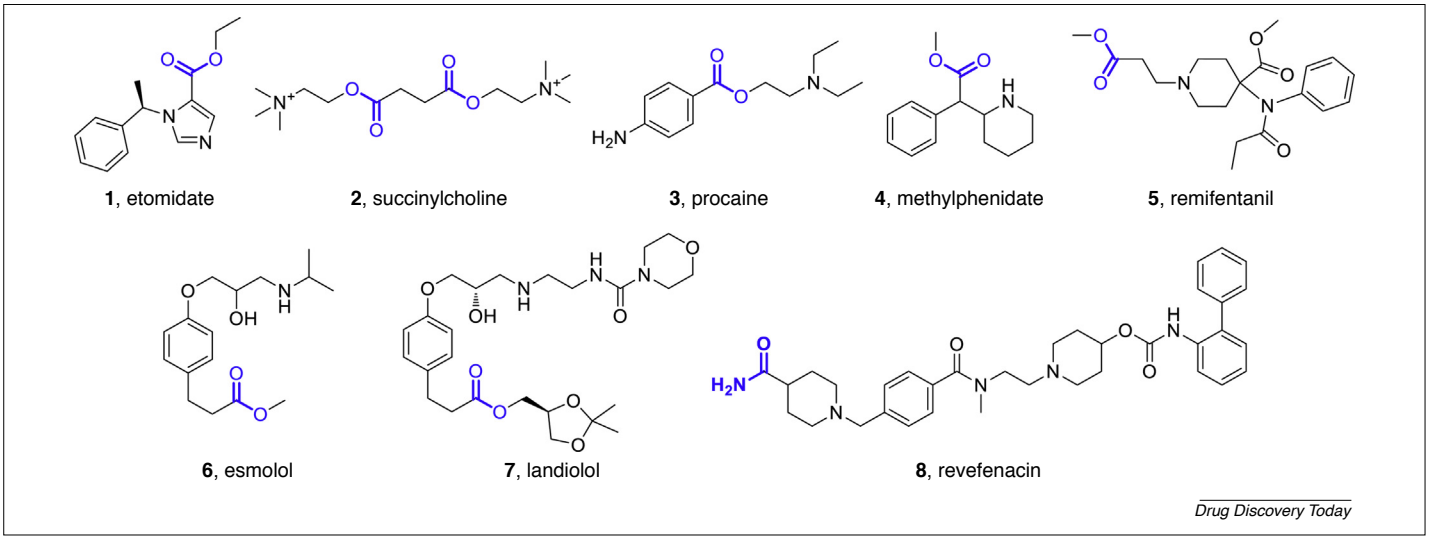


FIGURE 1

Illustrative examples of marketed soft drugs (SDs). Metabolically labile 'soft spots' are highlighted in blue.

total skin surface area (1.5–2 m^2, 15% of the body weight) [13]. Given that metabolism also impacts dermal absorption, metabolic activation (e.g., genotoxicity and sensitization induced by electrophilic reactive species), and skin irritation (e.g., conversion of esters into irritating alcohols, which are further oxidised to aldehydes and carboxylic acids) [14], it is fundamental to understand which are the contributing enzymes in the skin, because they are different from the systemic metabolism.

The approaches used to characterise cutaneous metabolism in recent years have made use of mRNA expression, proteomic profiling [15], protein expression, and probe substrate activity [14,16]. Although the presence of mRNA, which encodes a specific metabolising enzyme, is an indication that the protein might be synthesised in the skin, this does not always correlate with the presence of the respective protein or with its functional activity and, therefore, a conclusive picture of the enzyme activity in the skin is still required. Nevertheless, it is well established that weak cytochromes P450 (CYP)-dependent monooxygenase, high transferase and esterase activities characterize the cutaneous setting, pointing to skin as a detoxifying, rather than an activating, organ [13].

CYPs of the families 1–3, which are the major contributors to metabolism in the liver, show low activities in skin, with a decreasing order of potency in epidermis, dermis, and whole skin, indicating that their main localisation is in keratinocytes compared with other cell types [16]. Flavin-containing monooxygenases (FMOs) and cyclooxygenases (COXs), as well as other oxidoreductases, including xanthine oxidase and peroxidases, have been reported to be the non-CYP oxidoreductases of potential relevance for the skin [14]. The presence of aldehyde oxidase (AO) was only recently described, showing remarkable reaction rates [17].

The most important phase II enzymes in skin are glutathione *S*-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), *N*-acetyltransferases (NATs) [18], and sulfotransferases (SULTs), the latter possibly leading to toxic metabolites (e.g., severe immune-mediated skin rash mediated by reactive benzyl sulfate) [19].

Hydrolysing activity is reported to be relevant in skin [13]. Among the different hydrolases, esterases and/or amidases are known to contribute significantly to drug metabolism [20]. Figure 2 exemplifies the main compartments involved in the ester hydrolytic inactivation of topical SDs and their corresponding enzymes, together with the *in vitro* tools useful in the assessment of the softness degree.

Carboxylesterases (CEs) catalyse the hydrolysis of esters, amides, thioesters, and carbamates. Human carboxylesterases (hCEs) are highly expressed at the cutaneous level [13], but investigations into the expression of specific hCEs isoforms in skin remains controversial [21]. Indeed, both Zhu and Fu examined the expression of hCEs in HaCaT cells and detected hCE2 mRNA, whereas the hCE1 counterpart was undetectable [22,23]. By contrast, a more recent proteomic analysis of human skin highlighted the sole presence of hCE1 isoform [15]. CEs have a different propensity regarding the nature of substrates: it is well known that the preferential substrates for hCE1 are esters displaying a less hindered alcohol counterpart, whereas those for hCE2 are esters bearing a bulkier one [24]. Curiously, CEs are present in the plasma of most rodents, but are not found in primates [25]. By contrast, human plasma contains butyrylcholinesterase (BChE), paraoxonase (PON1), and acetylcholinesterase (AChE). Albumin (HSA) displays only poor esterase activity, but is so abundant (~4%) [26] that it significantly contributes to ester hydrolysis [27]. Liver is characterised by the presence of CEs, with the hCE1 isoform more abundant than hCE2.

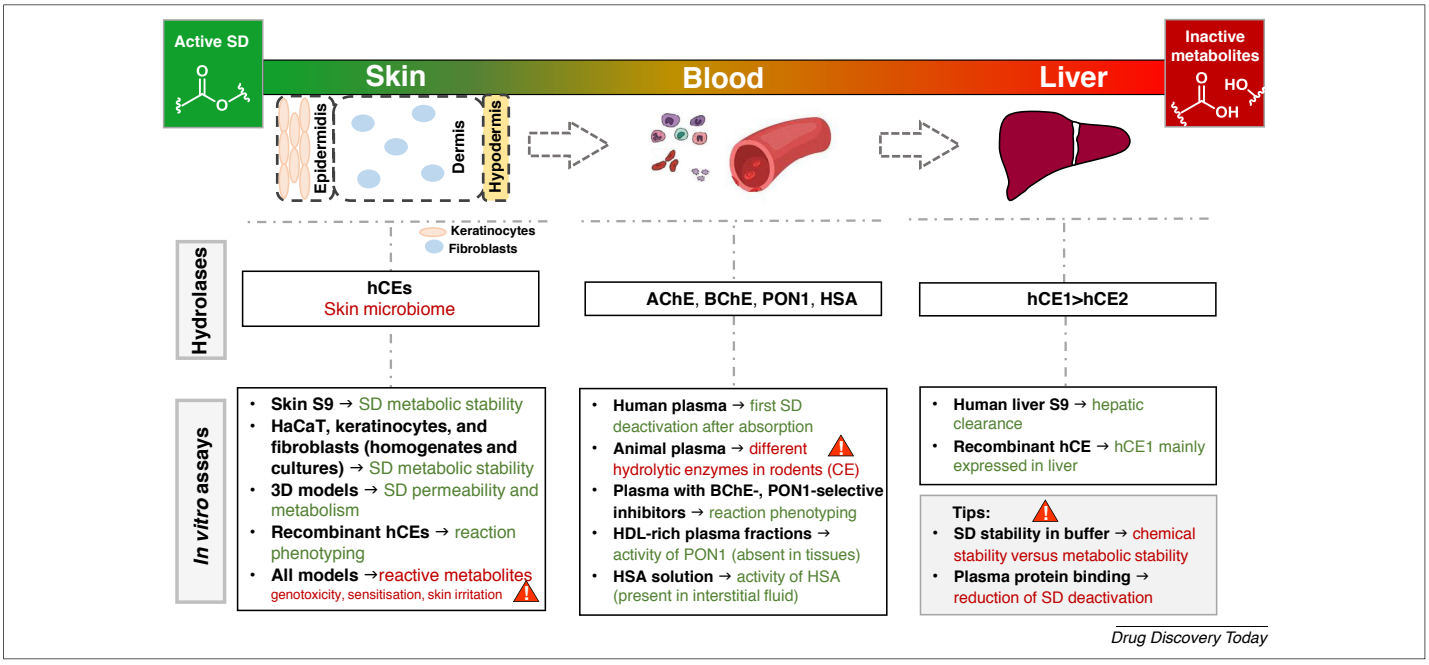


FIGURE 2

Hydrolytic metabolic inactivation of ester soft drugs (SDs): enzymes involved and *in vitro* tools used to assess it. Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; HaCaT, human skin keratinocytes cell line; hCEs, human carboxylesterases; HDL, high-density lipoprotein; HSA, human serum albumin; PON, paraoxonase.

Pathological conditions of the skin can affect the quality and extent of metabolism. Examples reviewed by Zhang *et al.* [28] show that significant induction of oxygenases and GSTs might occur in psoriasis, whereas the gene expression decrease for some CYPs. Surprisingly, polymorphisms of *CYP1A1* have also been shown to be associated with acne, although the relationship between xenobiotic metabolism and disease has not been extensively investigated [28].

The human skin harbours >200 bacterial genera, with up to 10 million bacterial cells/cm^2, a microbiome that is unique compared with other mammals [29]. The skin microbiome is capable of metabolising drugs and, therefore, might represent an important source for the metabolic activation and inactivation of xenobiotics [28,30]. This is even more true with regard to infected and/or diseased skin.

Tools to assess skin metabolism

The most reliable experimental approach to assess drug metabolism in human skin is the use of viable *ex vivo* human skin samples. Indeed, topical application of the substrate on these models allows the evaluation of both skin permeation and metabolism. However, obtaining fresh *ex vivo* skin is far from straightforward and is often limited by the availability of donors.

The most practical *in vitro* systems include the use of skin S9 homogenates derived from epidermis of abdominal skin, cultures of human primary keratinocytes and immortalized cells (e.g., immortalized human keratinocytes; HaCaT) and recombinant hCEs [14].

Since 2013, when the use of animal testing was banned for testing novel cosmetic ingredients, innovative human 3D skin models have been developed as an alternative to animal experimentation. Examples include: (i) EpiDerm^{TM}, a 3D multilayered skin culture derived from human neonatal foreskin keratinocytes; (ii) Episkin^{TM}, a reconstructed human epidermis model derived from female adult keratinocytes from mammoplasty; (iii) reconstructed human epidermis (RHE), normal human keratinocytes derived from human neonatal foreskin; (iv) full-thickness models (FTMs), a dermal equivalent with human fibroblasts overlaid by a stratified, well-differentiated epidermis derived from normal human keratinocytes cultured on an inert polycarbonate filter (see [13] for histology and metabolising enzyme activity); and (v) a root sheath-derived reconstructed human epidermal (ORS-RHE) [14,31]. With respect to skin homogenates and keratinocytes cultures, reconstructed 3D models have a barrier that includes fibroblasts embedded in collagen, mimicking the dermis layer of natural skin. Moreover, the comparison of the metabolising activity of some 3D skin models with *ex vivo* human skin showed weak monooxygenase activity for both the systems, alongside high esterase and transferase activities [13], thus suggesting the usefulness of these models for predicting SD stability in skin. Bätz *et al.* [32] reported that RHE and FTMs might be useful to quantitatively address the esterase activity of human skin in drug development, although increased activity compared with native human skin must be taken into account. Expression and activity of GSTs and NATs were found to be similar in both normal human skin and skin models [13].

Illustrative examples of dermatological SDs

Over the years, the SD principle has been used to target a plethora of pathways involved in the pathogenesis of skin diseases; here, we present an overall review of examples, highlighting the rationale behind the SD design. In Table 1, the most characterized compounds are reported, together with the biological activity in comparison with the metabolite. The models used for the assessment of the softness of the described compounds are detailed, when available, together with the clearance values and the experimental conditions used for their evaluation.

Steroids

Corticosteroids are among the most effective drugs on the market, but are burdened by severe adverse effects, even when applied topically. Therefore, it is not surprising that the SD principle has been extensively used in this field with the aim to improve the risk–benefit ratio.

The development of soft corticosteroids represents the first rational application of the SD principle, leading to the successful development of both loteprednol etabonate (LE), [34] currently approved by US Food and Drug Administration (FDA) for the treatment of ocular inflammation, and etiprednol dicloacetate (ED) [35], another soft corticosteroid that completed a Phase II clinical study but was not developed further [36]. These two examples are not dermatological drugs, but warrant a closer look because they bring to light important aspects for SD design.

LE and ED were both designed starting from prednienic acid, an inactive metabolite of prednisolone. In particular, LE displays an ethyl carbonate ester and a chloromethyl ester (**9**, Fig. 3), whereas ED has a dichloromethyl ester and an ethyl ester at 17α- and 17β-positions (**10**, Fig. 3). Recently, it was reported that, although being stable in human liver and intestinal S9 fractions, LE in human plasma is slowly hydrolysed to a carboxyl metabolite at position 17β, whereas ED is metabolized, tenfold faster, to the hydroxylated derivative at position 17α (Table 1) [26], indicating that the dichloroacetyl function is cleaved more easily than the hindered 17β-ester.

Hydrolysis assessment in the presence of selective inhibitors of plasma esterases (paraoxon and eserine) or with HSA showed that the main enzyme responsible for the hydrolysis of LE and ED is PON1, whereas HSA slightly hydrolysed ED but not LE. Given that PON1 is active in plasma, whereas hCEs are abundant in several tissues, LE and ED are stable in several districts (i.e., in the site of application), but they are promptly inactivated in plasma, a behaviour that is considered ideal for SDs. Furthermore, PON1 is associated with high-density lipoprotein (HDL), which does not diffuse from blood into tissues, guaranteeing the stable presence of LE at the administration site. By contrast, ED might be inactivated by HSA, which is instead present in the interstitial fluid of the administration site. In addition to the inactivation in plasma, the strong protein binding (98%) shown by LE and ED minimises their systemic adverse effects, confirming that plasma protein binding must be considered for the correct evaluation of hydrolytic stability data in plasma.

Fluticasone propionate, an alleged soft steroid, does not undergo hydrolysis at the 7 β-fluoromethyl thioester because its clearance depends on the relatively slow CYP-450 catalysed metabolism [37]. In another study exploring analogs of ED [38], the dichloroacetyl function was inserted at position 17α and the resulting compound (**11**, Fig. 3) was endowed with anti-inflammatory activity (IC_{50} = 3.0 nM, Table 1), measured as the ability to inhibit tumour necrosis factor (TNF)-α production in lipopolysaccharide (LPS)-stimulated blood cells. This activity decreased both in diluted human blood (1:5) and after overnight preincubation in human

TABLE 1

Available data for the most characterized SDs discussed in the review[a]

Compound	Class	Phase of development[b]	Enzymatic reaction	Activity SD/metabolite	Model for softness assessment	Clearance	Experimental[c]	Refs
9, LE; **10**, ED	Steroids	LE: approved ED: clinical	Hydrolysis (LE and ED)	LE: K_D 4.39 nM/N.D. ED: IC_{50} (nM) 73[d]/N.D.	hLS9	2.04 (LE), n.d. (ED) pmol/min/mg; 0.21 ml/h/kg (LE)[e]	s = 20 μM, p = 100 μg/ml	[26,33,34,38]
					hIS9	10.1 (LE), n.d. (ED) pmol/min/mg	s = 20 μM, p = 100 μg/ml	
					hPL	$t_{1/2}$ = 12.2 h (LE), 1.35 h (ED); 2.41 ml/h/kg (LE)[e]	s = 150 μM[f]	
					HSA	$t_{1/2}$ = 69.4 h (LE), 7.14 h (ED)	p = 4% HSA solution	
11	Steroids	Preclinical	Hydrolysis	IC_{50} (nM) 3.0[d]/N.D.	hPL	1[g]	s = 5 ng/ml	[38]
12	Steroids	Preclinical	Hydrolysis	IC_{50} (nM) 6.4/400	hPL	$t_{1/2}$ = 5 min	s = 30 μg/ml	[40]
					Human lung S9	stable ≥2.5 h	s = 50 μg/ml	
14, FP16CM	Steroids	Preclinical	Hydrolysis	IC_{50} (nM) 50.3/N.D.	rPL	$t_{1/2}$ = 29.4 min	s = 30 μg/ml	[44]
15	Steroids	Preclinical	Hydrolysis	Ki_{ER} (nM) 6.8/N.D.	rLM	RHA[h] = 420	s = 50 μM, p = 0.28 mg/ml	[46]
17, sofpironium bromide	Anticholinergics	Clinical	Hydrolysis	*pKi* M1–M4 8.99–9.45/6.43–8.33	Rat blood rPL	k = 7.2 min^{-1}, $t_{1/2}$ = 96.6 min (k = 8.0 min^{-1}, $t_{1/2}$ = 86.4 min)[j]; k = 15.8 min^{-1}, $t_{1/2}$ = 44.0 min (k = 20.9 min^{-1}, $t_{1/2}$ = 33.8 min)[j]	s = 700 μM	[50]
18, crisaborole	PDE4 inhibitors	Approved	Oxidation and hydrolysis	IC_{50} PDE4 (nM) 490/N.D. (**18**)	–	–	–	[58]
20	PDE4 inhibitors	Preclinical	Hydrolysis	$IC_{50\ PDE4}$ (nM) 47 /inhibition at 10 μM 5%	mPL *in vivo* (mice) sc, po, iv	27.5% remaining after 1 h	s = 5 μM	[60]
					In vivo (mice) sc, po, iv	$t_{1/2}$ = 1.03 h (iv)	100 mg/kg (sc, po), 25 mg/kg (iv)	
21, lotamilast (E6005, RVT-501)	PDE4 inhibitors	Clinical	Hydrolysis	IC_{50} (nM) 0.49–3.1/>100[i]; IC_{50} PDE4 (nM) 2.8/N.D.	–	–	–	[62]
22, LEO-29102	PDE4 inhibitors	Clinical	Hydrolysis	IC_{50} PDE4 (nM) 5/3760	hLM	CL = 200 ml/min/kg	s = 0.5 μM, p = 0.5 mg/ml	[66]
26	S1PR1 modulators	Preclinical	Hydrolysis and conjugation with UDPGA	IC_{50} S1PR1 (nM) 25/10 (**27**)/0 (**28**)	hSS9	$t_{1/2}$ = 7.3 min	s = 0.5 μM, p = 0.3 mg/ml	[72]
29	TRPV1 modulators	Preclinical	Hydrolysis	IC_{50} TRPV1 (nM) 930/residual activity @ 1 μM 6.7%	hCE1	$t_{1/2}$ = 27.2 min CL = 118 μl/min/mg	s = 50 μM, p = 0.2 mg/ml	[76]
					hCE2	$t_{1/2}$ = 12.5 min CL = 256 μl/min/mg	s = 50 μM, p = 0.2 mg/ml	
					hLS9	Totally depleted after 1 h	s = 50 μM, P = 1 mg/ml	
					hPL	82.0% remaining after 1 h	s = 100 μM	
					HaCaT homogenate	98.6% remaining after 4 h	s = 25 μM, p = 0.4 mg/ml	
					Keratinocyte homogenate	22.6% remaining after 2 h	s = 50 μM, p = 0.4 mg/ml	
					Fibroblast homogenate	13.3% remaining after 2 h	s = 50 μM, p = 0.4 mg/ml	
30	JAK inhibitors	Preclinical	Conjugation with UDPGA (putative soft spot)	IC_{50} JAK1, 2, 3 (nM): 4.4, 6.3, 3.2/N.D.	hLM	CL = 47 ml/min/kg	–	[80]
31, PF-06263276	JAK inhibitors	Clinical	Conjugation with UDPGA	IC_{50} JAK1, 2, 3, and TYK2 (nM): 2.2, 23.1, 59.9, 29.7/N.D.	hLM (Phase I)	CL = 101 μl/min/mg	–	[83]
					hLM supplied with UDPGA (Phase II)	CL = 129 μl/min/mg	–	
32	Caspase 1 inhibitors	Preclinical	Hydrolysis (putative soft spot)	IC_{50} THP1 (nM) 38/N.D.	hSS9	CL = 0.92 μl/min/mg	s = 1 μM, p = 2.3 mg/ml	[85]
					Normal human epidermal keratinocytes	CL = 4.6 μl/min/10^6 cells	s = 1 μM, 70 × 10^3 cells per well	
					Human hepatocytes	CL = 9.8 μl/min/10^6 cells	s = 1 μM, 0.5 × 10^6 cells/ml	

[a] Abbreviations: hIS9, human intestinal S9; hLS9, human liver S9; hPL, human plasma; rPL, rat plasma; rLM, rat liver microsomes; mPL, mouse plasma; hLM, human liver microsomes; hSS9, human skin S9; hCE1–2, human carboxylesterase 1–2.
[b] Phase of development represents the most advanced phase reached.
[c] s = substrate concentration; p = enzyme protein concentration.
[d] Inhibition of TNF-α production of LPS-stimulated whole blood cells.
[e] Estimated clearance based on scaling factors.
[f] Hydrolysis rate determined at increasing substrate concentration up to 150 μM.
[g] Chromatographic peak area respect to the internal control.
[h] RHA = relative hydrolytic activity compared to E16-1,2 = 100.
[i] Suppression of cytokine production.
[j] Data refer to compound **17** (***2R*** enantiomer).

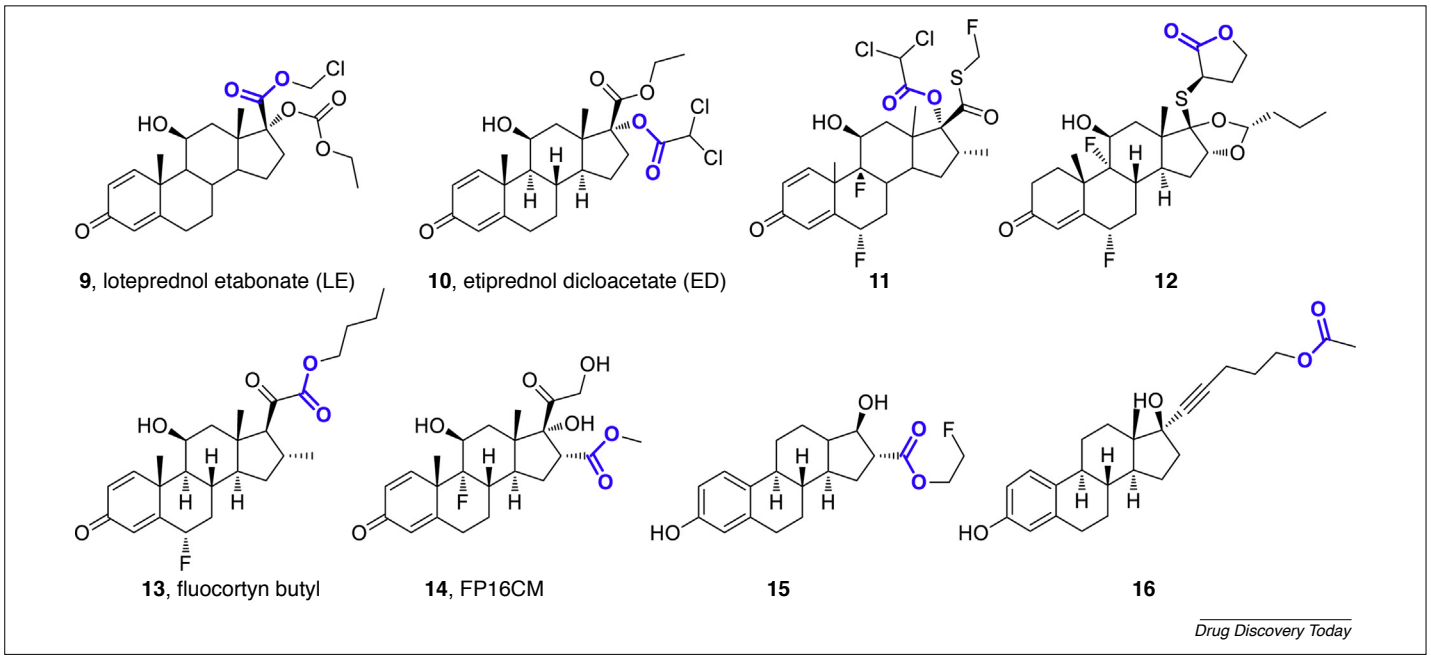


FIGURE 3

Soft steroids: representative examples. Metabolically labile 'soft spots' are highlighted in blue.

plasma, suggesting low hydrolytic stability in systemic circulation, even though the enzymes responsible for the deactivation are not identified [38]. Moreover, the anti-inflammatory activity of compound **11**, when intratracheally administered in ovalbumin-sensitised Brown Norway rat model, was superior to that of fluticasone.

PONs are enzymes also responsible for the inactivation of different series of glucocorticoids described by Procopiou *et al.* [39–41] in an extensive medicinal chemistry program aimed at discovering safer corticosteroids. The best candidate identified displayed a lactone moiety (**12**, Fig. 3) that is rapidly hydrolysed in plasma (Table 1) to give an inactive metabolite (IC_{50} = 400 nM versus IC_{50} = 6.4 nM of **12**), while being stable in the lung S9 fraction for at least 2.5 h (Table 1). It had a topical anti-inflammatory activity in the rat ear oedema model, showing attenuated adverse effects in the thymus involution test. These properties suggest such derivatives as excellent candidates for their topical use in asthma, but no further development has been reported.

While the aforementioned examples refer to the treatment of ocular or respiratory diseases, fluocortyn butyl (**13**, Fig. 3) is a corticosteroid contained in marketed anti-inflammatory dermatological creams. It is the butyl ester of a C_{21} carboxy steroid and was designed starting from fluocortolone-21-acid, an inactive metabolite of fluocortolone [42]. Fluocortyn butyl has the advantage of being hydrolysed into inactive species when systemically absorbed, but a full characterisation of its softness remains missing from the literature and its low intrinsic activity has so far limited its use.

Over the years, several groups have attempted to further exploit the SD principle in the corticosteroid arena. Lee and collaborators have been active in the development of anti-inflammatory agents and their efforts in this direction have already been reviewed elsewhere [43]. Among the synthesised prednisolone derivatives displaying esters at various positions, FP16CM, a 9α-fluoroprednisolone with a methyl ester at C-16 (**14**, Fig. 3), was shown to be able, on the one hand, to reduce the inflammation in a croton oil-induced ear oedema model and, on the other hand, to limit undesired effects, such as body weight gain, thymus atrophy, or suppression of endogenous corticosterone levels. These features were attributable to the hydrolysis of FP16CM ($t_{1/2}$ = 29.4 min in rat plasma, Table 1) into the inert acid metabolite. A hydrolysis study showed that a benzyl ester derivative (FP16CB), although endowed with a minor activity, was hydrolysed quickly ($t_{1/2}$ = 3.2 min) [44].

Similar local anti-inflammatory activity and reduced systemic adverse effects were reported in 2014 for β-methylphenethylamine (MPEA), a cortienic acid derivative displaying both an amide and an ester in the chain at position 17β; however, only *in silico* predictions of metabolism and the biological activity of the putative main metabolite have been reported, thus limiting the significance of this research [45].

Besides corticosteroids, the SD principle has been applied to other soft steroids, including estrogens, with the aim of developing agents able to perform an estrogenic stimulation confined to the area of administration [46]. Given that the skin contains estrogen receptors (ER), it represents a target organ for estrogen replacement therapy, and topical application of estrogens has been used to treat vaginal dyspareunia of menopause. Nevertheless, estrogen administration is associated with several risks, such as endometrial cancer, breast cancer, and stroke; therefore, the development of a soft topical agent would offer a means of improving safety, especially in women for whom systemic estrogens are contraindicated. A series of estradiol-16α-carboxylic acid esters were synthesized and evaluated for their ER affinity, for the biological activity in an *in vitro* estrogen-sensitive model, and for the relative rate of their enzymatic hydrolysis in rat hepatic

microsomes [46]. The 2′-monofluorethyl ester of E_2-16α-formate (**15**, Fig. 3) appeared the most promising compound, with a relative binding affinity (RBA) for human ERα of 16 and ERβ of 0.1, whereas the corresponding acid metabolite E16-1,0 was devoid of activity (RBA = 0). The high rate of hydrolytic cleavage (Table 1) explains why, in the *in vivo* uterotrophic test for the evaluation of systemic estrogenic activity, the compound administered by subcutaneous injection was almost inactive, whereas, in the *in vivo* vaginal assay for the evaluation of local estrogenic activity, after topical application the compound exerted a significant estrogenic action. Two years later, the same authors reported carboxylic acid esters of estradiol at the α-, 11β- and 15α-positions. Among them, two compounds, the methyl and ethyl esters of estradiol-15α-formate, were shown to exert an improved local to systemic estrogenic profile [47]. Capitalising on the well-known beneficial effects of estrogens on cutaneous wound repair, 17α-substituted estradiol derivatives bearing an ester moiety were also investigated. One compound (**16**, Fig. 3), when injected subcutaneously, accelerated repair in a murine model of age-associated delayed wound healing at the same level as 17β-estradiol, without exerting systemic activity on ER [48].

Other soft dermatological agents

Although the SD concept has primarily focused on the discovery of safer corticosteroids, over the past decades, the scope has broadened to involve other therapeutic classes (Table 1).

Anticholinergics

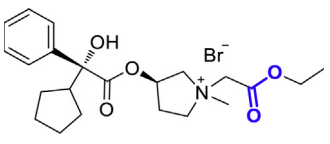
Topical anticholinergics find application as mydriatic and/or cycoplegic agents, as well as antiperspirants, but might lead to systemic toxicity. The SD principle has been used since 1980 [5,49] starting from different lead compounds (i.e., acetylnorcholine and propantheline) and leading to the development of several series of soft anticholinergics, with the aim of separating the local therapeutic benefits from the off-site toxic effects. These attempts led to the recent clinical success of sofpironium bromide (BBI-4000, **17**, Fig. 4) for the treatment of hyperhidrosis, an unmet medical need. It was designed starting from glycopyrrolate, a quaternary ammonium compound with poor blood–brain barrier permeability and reduced central nervous system (CNS)-related adverse effects. In particular, one of the methyl groups on the quaternary ammonium site was replaced with an alkoxycarbonylethyl function and the resulting ester was found to be susceptible to extrahepatic metabolism (rat blood and plasma), consistent with its soft nature [50,51]. The fast hydrolysis to the less active zwitterionic metabolite resulted in a decrease of typical peripheral anticholinergic adverse effects (i.e., urinary retention and visual disturbances) [52]. For these reasons, sofpironium bromide has entered clinical trials and has completed Phase 2IIb and a Phase III trial in Japan, with a Phase III trial starting in the US [53–55].

Interestingly, the same authors reported that the derivative displaying the methyl ester instead of the ethyl ester was not hydrolysed by CESs, ascribed to the fact that the quaternary ammonium structure impairs the transport into the luminal side of the endoplasmic reticulum, where CESs are located. By contrast, the hydrolysis is mediated by PON1. A difference, albeit small, in terms of hydrolytic stability is reported between rat (half-life: 9.8 min) and human (half-life: 16.9 min) plasma and this correlates with the different expression level of the enzyme in the two species.

PDE4 inhibitors

PDE4 has emerged as a promising target for the treatment of various diseases associated with inflammation and, over the past decades, a plethora of inhibitors has been developed, leading to the approval of three drugs (i.e., roflumilast, apremilast, and crisaborole) in different clinical settings (airway diseases, psoriasis, and atopic dermatitis, respectively) [56]. However, significant efficacies of PDE4 inhibitors are often accompanied by serious adverse effects, especially on the gastrointestinal tract (nausea and vomiting). Roflumilast and apremilast were approved for oral use in 2010 and 2014, respectively, but both show non-negligible systemic impact, including psychiatric disorders. Topical delivery, such as inhalation and cutaneous application, would mitigate these adverse effects [57].

This was exemplified by the successful development of crisaborole (AN2728, **18**, Fig. 5) [58], a topical benzoxaborole approved in 2016 in the US for adults and children older than 2 years with atopic dermatitis (IC_{50} = 490 nM). Its low molecular weight provides skin penetration, whereas the benzoxaborole geometry mimics the phosphate of cAMP. Clinical trials demonstrated that 2% ointment applied twice daily alleviated symptom severity, without causing gastrointestinal adverse effects [59]. Crisaborole penetrates the epidermis and dermis, and, when it reaches the systemic circulation, it is extensively metabolised into inactive metabolites, avoiding systemic exposure. The major metabolite is 5-(4-cyanophenoxy)-2-hydroxyl benzyl alcohol (AN7602, **19**, Fig. 5), which is formed by oxidative deboronation and subsequent hydrolysis. Given that the first metabolic step involves an oxidation mediated by CYP, crisaborole is not a canonical SD. AN7602 is further metabolised to produce several downstream metabolites, among which 5-(4-cyanophenoxy)-2-hydroxyl benzoic acid (produced by oxidation) and AN7602-sulfate (produced by sulfation) are major circulating components. CYP3A4 and 1A1/2 appear to have a major role in the formation of AN7602, together with CYP2B6 and 2E1, which also appear to contribute.



17, sofpironium bromide

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FIGURE 4

Sofpironium bromide. The metabolically labile 'soft spot' is highlighted in blue.

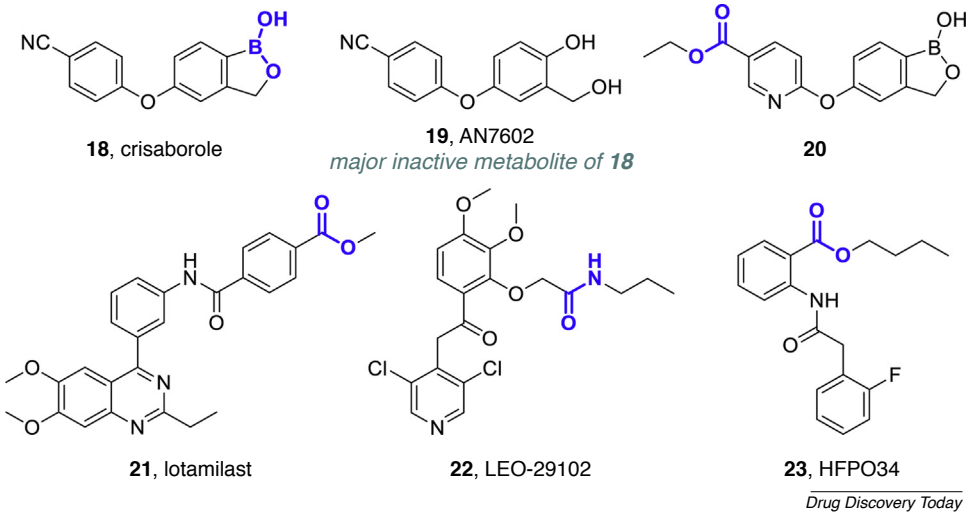

FIGURE 5

Representative soft phosphodiesterase 4 (PDE4) inhibitors. Metabolically labile 'soft spots' are highlighted in blue.

Starting from crisaborole, the same authors developed a series of benzoxaborole derivatives where the soft spot was represented by the ester moiety. Among these derivatives, one compound (**20**, Fig. 5) displayed an IC_{50} of 47 nM and a high tendency to be hydrolysed *in vitro* in mouse plasma and *in vivo* in mice to the corresponding and inactive acid metabolite (Table 1). It inhibited ear oedema caused by phorbol esters after dosing at 1 mg/ear twice, suggesting a good skin stability and significant *in vivo* anti-inflammatory activity. It showed also a reduced emetic activity [60].

Two PDE4 inhibitors are currently in Phase II for atopic dermatitis. The first is lotamilast (**21**, E6005, RVT-501, Fig. 5) [61], which inhibits PDE-4 with an IC_{50} of 2.8 nM. In mice models, topical application improved skin inflammation and pruritus without serious adverse effects [62]. Given that it displays a methyl ester, lotamilast is rapidly metabolized to the carboxylic acid (100 times weaker in suppressing cytokine production) and was characterised by a rapid clearance from the blood system and low distribution to the brain, as demonstrated by using ^{14}C-labeled lotamilast in mice [62]. Data collected in humans confirmed the preclinical data [63,64] and explained the very low levels of nausea and vomiting reported.

The second candidate is LEO-29102 (**22**, Fig. 5) [65], a compound displaying an IC_{50} value of 5 nM. It was well tolerated in preclinical models with no emetic episodes or other prodromal signs up to 1 mg/kg, whereas the pharmacokinetics study in humans supported the SD concept, obtaining low systemic levels of active compound (C_{max} 0.279–5.07 ng/ml and $AUC_{0\text{-}48h}$ 5.45–100 ngh/ml, according to the treated body surface area). The soft spot is represented by the amide, which, in human liver microsomes, undergoes fast hydrolysis (Table 1), resulting in the corresponding carboxylic acid being the primary and inactive metabolite (IC_{50} = 4 μM) [66].

HFP034 (**23**, Fig. 5) is an anthranilate derivative with an IC_{50} value of 4.2 μM. In an imiquimod-induced mouse psoriasis model, its topical application ameliorated skin lesions and epidermal thickness. It is characterised by a butyl ester and the authors, using an *in vitro* Franz cell, demonstrated that the compound accumulates in the skin and is not delivered into systemic circulation. Although it is not described as a SD and no investigation on its metabolism has been reported, it is reasonable to assume that the compound is hydrolysed by esterases [67].

S1PR1 modulators

Besides PDE4 inhibitors, S1PR1 agonists have recently received widespread attention in the search for antipsoriasis treatments [68]. These compounds activate S1PR1 and lead to the internalization of the receptor, preventing the maturation and the migration of lymphocytes with an overall immunosuppressive effect. To date, fingolimod is the only S1PR1 agonist that has received FDA approval for the treatment of relapsing forms of multiple sclerosis, an indication extended also to the paediatric population in 2018. Ponesimod, another S1PR1 agonist, reached Phase II clinical trials for severe plaque psoriasis [69,70], but has not proceeded further. Indeed, this class of molecules is affected by severe adverse effects (i.e., lymphopenia, bradycardia, and dyspnoea) that hamper its use in dermatological conditions. Starting from the structure of ponesimod, Bell *et al.* [71] reported a first series of ester analogues displaying good activity on S1PR1, with an IC_{50} value of 2.5 nM for the most potent one. Compound **24** (Fig. 6) is hydrolysed to an inactive metabolite ($IC_{50} > 1$ μM), but is relatively stable in human plasma, with a half-life of 180 minutes, compromising its soft nature. Further optimisation led to the identification of compound **25** (Fig. 6), with improved solubility and short half-life in both human plasma and S9 skin fraction (9 and 23 min, respectively, Table 1). Unfortunately, the compound does not display significant activity on S1PR1 ($IC_{50} > 1$ μM). In a second, more successful attempt, the authors described ponesimod analogues [72] in which the soft spot is provided by the phenol moiety. The most potent compound (**27**, Fig. 6) displays an IC_{50} value of 10 nM on S1PR1 and intrinsic clearance in human liver microsomes and human liver hepatocytes of 2.8 ml/min/g and 31 ml/min/g, respectively. It is rapidly metabolised by Phase II enzymes in human hepatocytes to give **28** (Fig. 6). To avoid the chemical instability related to the phenol group, the hydroxyl moiety was esterified to give the methyl ester **26** (Fig. 6). The resulting molecule retains good potency on S1PR1 (IC_{50} 25 nM) and is rapidly converted into **27** by the hydrolysing activity of skin S9 fraction esterases (Table 1). Compound **26** represents a peculiar example of a SD that requires two steps for its inactivation: hydrolysis in skin and conjugation with glucuronic acid in liver.

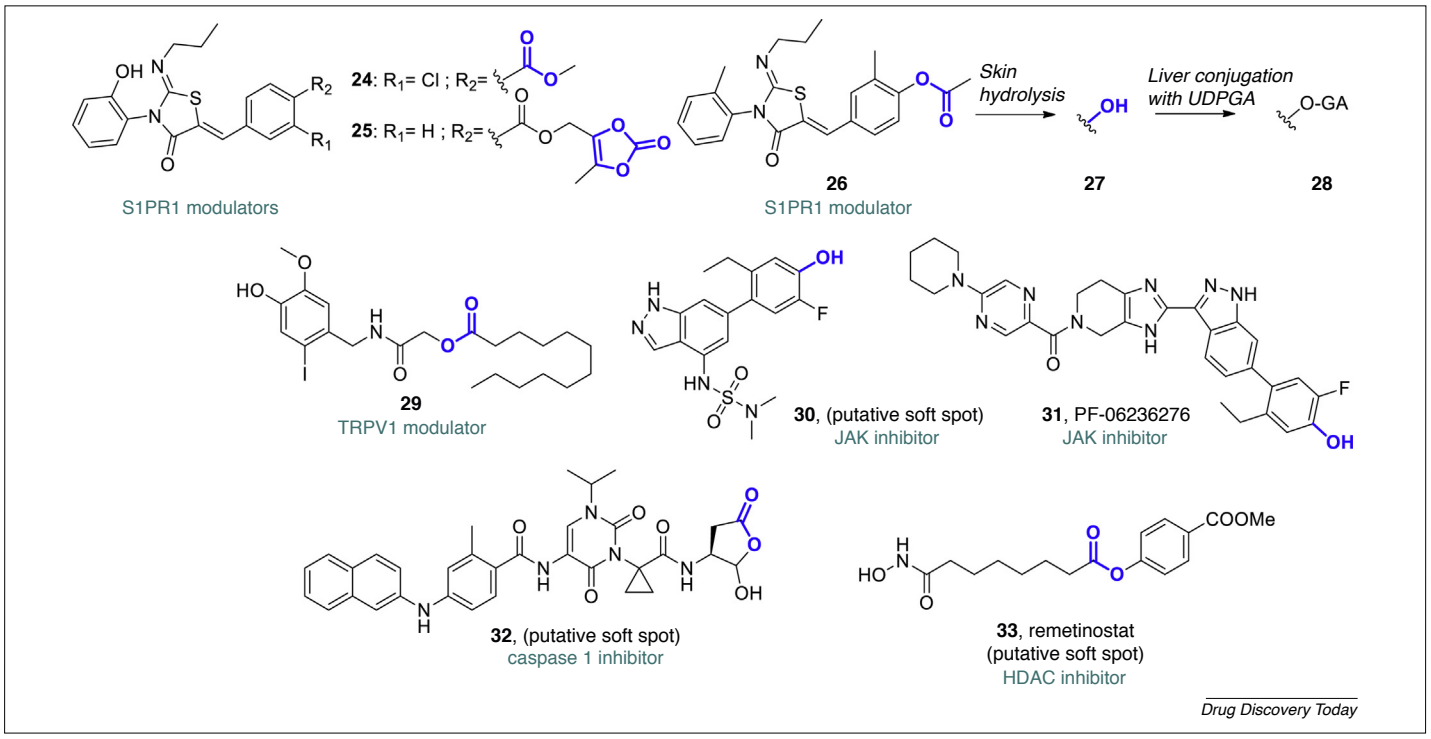

FIGURE 6

Other soft dermatological agents. Metabolically labile 'soft spots' are highlighted in blue. Abbreviations: HDAC, histone deacetylase; JAK, Janus kinase; S1PR1, sphingosine-1-phosphate receptor 1; TRPV1, transient receptor potential vanilloid 1; UDPGA, uridine diphosphate glucuronic acid.

TRPV1 modulators

In 2018, the SD principle was applied to the structure of capsaicin, the pungent principle of hot chili peppers. Capsaicin acts as agonist of the TRPV1 channel, a calcium permeable ion channel mainly expressed in peripheral sensory neurons, where the receptor acts as a mediator of cutaneous pain, inflammation, and itch. Beside its role in the nervous system, the channel is involved in the pathogenesis of skin disorders, especially related to inflammation and pruritus. The therapeutic potential of capsaicin-containing creams and patches is provided by the ability of the molecule first to open the channel, leading to the most common adverse effect, a so-called 'burning' sensation, and then to mediate its desensitisation. Capsaicin can reside in human skin relatively unchanged for a long period of time, leading to frequent erythema reactions. Moreover, it has been reported that chronic and long-term topical applications can increase the risk of skin carcinogenesis if co-applied with a tumour promoter, such as sunlight [73]. Besides agonists, topical antagonists able to block the channel have been reported, but many clinical trials have been interrupted because of the risk of hyperthermia induction and promotion of skin cancer [74,75]. To circumvent these adverse effects, a class of capsaicin soft analogues has been developed in which an ester moiety is inserted in the lipophilic tail [76–78]. This series included both agonists and antagonists, with a shift in the activity provided by the insertion of an iodine atom at 6-position of the aromatic core. Among these modulators, soft antagonists have been selected for further development, because no burning sensation was associated with topical administration. The most potent antagonist displays an IC_{50} value on TRPV1 of 90 nM, but a poor metabolic instability (unmodified in human plasma and 50% cleared in liver S9). Therefore, the lead compound selected for the *in vivo* evaluation was **29** (Fig. 6), which is endowed with a good balance of potency (IC_{50} 930 nM) and hydrolytic stability (Table 1). According to the SD principle, the hydrolytic metabolite is inactive on TRPV1 (6.7% of activity at 1 μM). Compound **29** is able to suppress TRPV1-mediated nociceptor excitability and reduces *in vivo* both thermal hyperalgesia and histaminergic and non-histaminergic mediated pruritus. The toxicity of the molecule has also been evaluated: it does not affect either thermal nociception or body temperature and does not induce any conspicuous behavioural or motor deficits or other discomfort, such as burning sensation and pain.

JAK inhibitors

JAKs, a family of four-member tyrosine kinases, represent a promising target in the field of inflammatory skin disorders, with several *in vivo* evidences of efficacy of JAK inhibitors in inflammatory disorders [79]. Two drugs, tofacitinib and ruxolitinib, have received FDA approval for the treatment of rheumatoid arthritis, and myelofibrosis and polycythaemia vera, respectively. Both drugs have been studied in several trials for the treatment of atopic dermatitis, psoriasis and alopecia aerata, but, because they are affected by important systemic adverse effects, such as immunosuppression and cytopenia, their use is incompatible with nonlife-threatening conditions.

In an attempt to reduce these adverse effects, a fragment-based screening was performed in 2016 with the aim to discover novel pan-JAK inhibitors for topical use [80]. The identified hit fragment displays an IC_{50} value of 30 μM on JAK1 and of 13 μM on JAK2. The optimisation of this fragment led to compound **30** (Fig. 6) with good potency on JAK1, JAK2, and JAK3 (IC_{50}: 4.4 nM, 6.3 nM, and 3.2 nM, respectively). Unfortunately, neither the structure of the main metabolites nor their lack of activity was assessed. The selected compound bears a phenol moiety as a putative soft spot, which

might undergo fast metabolism in the liver by conjugation with glucuronic acid, but no experimental data have been reported to support this hypothesis. Compound **30** displays an intrinsic clearance in human liver microsomes of 47 ml/min/kg (Table 1), high enough to be considered a SD compared with the liver blood flow rate (20 m/min/kg). From a toxicological point of view, **30** inhibits also other off-target kinases and displays significant phototoxicity related to the presence of the indazole moiety, which can be photodegraded to reactive or toxic degradation products, therefore making the compound a poor candidate for further development.

PF-06263276 (**31**, Fig. 6) is a pan-JAK inhibitor that advanced into clinical development [81,82] for psoriasis. A structure-based computational method allowed the discovery of a hit compound endowed with good potency on JAK1, -2, -3, and TYK2 (IC_{50} 0.4, 2.2, 8.3, and 9.1 nM, respectively), but affected by poor aqueous solubility and modest turnover for both Phase I and II metabolism (CL_{int} = 35 μl/min/mg hLM, and 49 μl/min/mg hLM, respectively supplied with uridine diphosphate glucuronic acid; UDPGA), despite the presence of a phenol moiety [83]. Further optimisation led to the identification of PF-06263276, which loses some potency on JAK1, -2, -3, and TYK2 (IC_{50} 2.2, 23.1, 59.9, and 29.7 nM, respectively), but displays an improved turnover both for oxidative (hLM) and conjugative (hLM supplied with UDPGA) clearance (Table 1). Moreover, the compound was selective for JAKs in a panel of 36 kinases and its crystalline solid form displayed excellent chemical and physical stability, allowing PF-06263276 to advance as a clinical candidate for both inhaled and topical delivery.

Caspase 1 inhibitors

In the pathogenesis of acne [84], many proinflammatory cytokines are involved and, in particular, interleukin (IL)-1β is abundant in human acne lesions. This cytokine normally exists in its inactive proform that, in the presence of inflammatory stimuli, is activated by the cleavage mediated by the aspartic cysteine protease caspase 1. A key binding element in caspase 1 inhibitors is a reactive warhead, which forms a covalent-reversible bond with the protein catalytic cysteine. Fournier *et al*. developed compounds in which an aspartyl aldehyde warhead is responsible for the activity, representing at the same time the soft spot because of its electrophilic nature [85]. The best candidate (**32**, Fig. 6, Table 1) shows high skin flux (4.3 μM/cm^2), good skin metabolic stability (CL_{int} of 0.92 μl/min/mg of protein in skin S9 fraction and a CL_{int} of 4.6 μl/min/10^6 cells in normal human epidermal keratinocytes) together with a good hepatic clearance (9.8 μl/min/10^6 cells in human hepatocytes) and low plasmatic exposure (1.3 nM). Nevertheless, no identification and evaluation of the resulting metabolites have been reported.

Androgen antagonists

Acne is also associated with the overproduction of sebum by the sebaceous gland. In this regard, soft androgen receptor antagonists have been described, relying not on hydrolysis, but on the oxidation of the thioether (IC_{50} = 43 nM) to the corresponding inactive sulfoxide (IC_{50} > 10 000 nM). A few were able to moderately reduce wax (43–66%) and cholesterol (35–50%) esters when topically applied in hamsters, but the authors did not report either the evaluation of the metabolic stability of these derivatives, or the reduction of the associated systemic adverse effects [86].

HDAC inhibitors

The SD principle is also being exploited in the oncological field, in particular in cutaneous T cell lymphoma (CTLC), a heterogeneous cluster of cancers that affect T cells in skin. Among the flourishing therapeutic strategies, HDAC inhibition is particularly relevant given their epigenetic modifying properties [87]. Vorinostat is a hydroxamic acid approved in 2006 as oral agent, whereas romidepsin is a cyclic depsipeptide approved by the FDA in 2009 for parenteral use. Their systemic activity is often associated with fatigue, nausea, diarrhoea, thrombocytopenia, and cardiac toxicity. Thus, to improve the tolerability profiles, the soft hydroxamic acid remetinostat (SHP-141, **33**, Fig. 6) was developed and has completed a Phase II trial for the treatment of early-stage CTLC [88–90]. After exerting its antitumoral activity in cutaneous lesions, it undergoes hydrolysis mediated by plasma BChE and PON1, to give methyl paraben and suberic acid hydroxamic acid as the main metabolites; however, further information is scarce and the methyl ester portion might be also hydrolysed [91]. Its topical formulation (SHAPE gel 0.5%), together with its hydrolytic lability, limit the adverse effects associated with systemic HDAC inhibition [92,93]. Preclinical examples of soft HDAC inhibitors include also a series of squaramides [94] characterised by high *in vitro* human hepatic clearance (29 μl/min/10^6 cells), although an evaluation of fulfilment of softness criteria has not been reported.

Discussion

Typical dermatological SDs are compounds that are sufficiently stable in the skin but are readily inactivated once they leave the cutaneous microenvironment. Most of the SDs reported so far have been discovered by designing close steric and electronic analogues of known active drugs (the so-called 'soft analogue approach' [12]), with the aim of retaining the biological activity, while controlling and directing the metabolism towards the most desired way. Therefore, once the SD is designed, it is necessary to verify that the introduction of the soft spot in the molecule retains the potency and that the main metabolites formed are inactive, an essential precondition for further SD development.

Most SDs are based on hydrolytic transformations, especially esterase-mediated reactions. Indeed, although drug clearance mainly relies on oxidative reactions mediated by CYP family, oxidation is rarely exploited in the design of SDs because it is generally slow, easily saturable, and often leads to reactive and toxic metabolites. The only examples in this review where softness relies on oxidation are crisaborole, **18**, where oxidative deborination occurs, and an androgen receptor antagonist where the thioether is oxidized to sulfoxide.

By contrast, hydrolysis reactions usually lead to inactive metabolites that are promptly conjugated and/or excreted. The loss of activity is usually attributed to a decrease in affinity to the target and/or to a reduced membrane permeability, both related to a decrease in lipophilicity. Moreover, hydrolysis reactions are fast and not easily saturable transformations. Compared with oxidations, which suffer from interspecies and interindividual variability, as well as inhibition and induction, hydrolysis is less prone to this variability, with few exceptions that include PON1, an enzyme affected by polymorphisms. Last, esterases are expressed in different levels, depending on the organ or tissue, and these differences could be exploited in the design of SDs that are stable in skin, but unstable in other settings.

Hence, it is not surprising that, in the examples discussed in this review, the insertion of a hydrolytically labile spot, an ester, or an amide, is the most pursued strategy in dermatological SDs design. Two exceptions are represented by SDs bearing a phenol that is conjugated with glucuronic acid (**30** and **31**), together with

compound **26**, where the methyl ester is first hydrolysed to the corresponding phenol (**27**) in the skin and then conjugated with glucuronic acid (**28**) in the liver [72,80,83].

SD stability in skin needs to be assessed and could be evaluated in a plethora of skin models, from skin S9 homogenates, to keratinocyte and fibroblast homogenates and cultures, alongside recombinant hCEs. 3D skin models have been so far overlooked, despite representing a reliable model for the assessment of both metabolism and permeation through the cutaneous barrier. By contrast, nonhuman models are not always predictive of the hydrolytic capacity of human skin, with primates being the best predictive model, although minipig skin subcellular fractions also appear a reliable model for quantitative human epidermal esterase activity [95]. Generally speaking, rodents have a higher hydrolytic activity compared with humans, whereas dogs have a slower hydrolytic activity. Therefore, in the preclinical development of a novel SD, undesirable ultrarapid hydrolysis in rodents does not necessary translate into a poor compound for human use.

Once it has exerted its therapeutic effects in skin, an ideal SD should undergo extensive metabolic inactivation before excretion, ideally first in bloodstream and then in other sites devoted to drug metabolism. Hence, SD inactivation in plasma and liver models needs to be investigated. Human plasma is endowed with high hydrolase activity mainly because of the presence of PON1 and BChE hydrolases. Given that they are almost absent in skin, PON1 and BChE are good candidates as bioconverting enzymes that can lead to successful SDs with effective local action and minimized systemic adverse effects, as exemplified by LE (**9**), ED (**10**), compound **12** [26,40], and sofpironium bromide **17** [52]. SD clearance in blood should be preferably assessed avoiding the use of plasma from mice and rats. Indeed, CEs are present in the plasma of most rodents but are not found in primates [25]. Besides differences in the expression level, phenotyping experiments with selective probes of plasma hydrolases (e.g., EDTA, an inhibitor of PON1, or eserine, a selective inhibitor of BChE) and incubations with HSA [26] enable researchers to predict the resistance of SDs at the site of action. Indeed, PON1, which is associated with lipoproteins, is not able to reach the administration site, in contrast to HSA, which is present in the interstitial fluid and in the administration site. Finally, plasma protein binding must be considered for the correct evaluation of the hydrolytic stability data in plasma. Indeed, if low levels of unbound drug in plasma occur, the hydrolysis rate of drugs in plasma should be considered to be faster compared with their hydrolysis in buffer, where drugs are totally unbound.

Structure–activity and structure–metabolism relationships (SARs and SMRs) should be exploited to reach a compromise between potency and softness degree and to identify the best candidate for *in vivo* proof of concept. The efforts in this direction could be reduced by relying on the information that is already available in the literature. For instance, it is well known that hydrolysis of linear alkanoates increases with the elongation of the chain, whereas branching and unsaturation generally decrease esterases activity [20,76]. Benzyl esters are labile toward hydrolases, [44,96] suggesting that they could be exploited for the design of very short-acting SDs. However, the differences in hydrolysis rate depend not only on the nature of the ester group, but also on its position. Altering both the nature and position of the ester moiety offers a means of modulating the hydrolytic stability profile of the compounds and covering a range of different potential indications for which local administration is applicable.

The experimental activities in this direction can be minimised using *in silico* techniques: virtual libraries of possible soft analogues could be generated and ranked according to the steric ad electronic analogy to the active lead compound and predicted hydrolytic lability [9,97–99].

Obviously, softness cannot be defined based solely on *in vitro* experiments, although *in vivo* data alone are insufficient for a fruitful drug discovery program not based on chance. Nevertheless, the literature is rich in examples where no efforts of SAR and SMR are undertaken and the SD is directly tested in an animal model to verify the local therapeutic activity and the absence of systemic adverse effects.

Concluding remarks

The challenge goal in SD design is not to accelerate metabolism, but rather to avoid it in the site where the action is desired (e.g., the skin), while maintaining it in the other compartments. The SD approach represents an effective tool in the medicinal chemists' toolbox, especially when safe, topically applied dermatological drugs need to be developed, but it is not necessarily straightforward. It is not a mere incorporation of an ester into an existing scaffold, and it requires the rigorous and experimental evaluation of a set of requirements. This is elegantly exemplified, for example, by recent work by Boland and coworkers [100] that describes soft ROCK inhibitors. Despite being beyond the scope of this review, because it does not target the skin, this study represents an excellent example of medicinal chemistry efforts made to develop a promising SD.

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