

# Demystifying racemic natural products in the homochiral world

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**Abstract** | Natural products possess structural complexity, diversity and chirality with attractive functions and biological activities that have significantly impacted drug discovery initiatives. Chiral natural products are abundant in nature but rarely occur as racemates. The occurrence of natural products as racemates is very intriguing from a biosynthetic point of view; as enzymes are chiral molecules, enzymatic reactions generating natural products should be stereospecific and lead to single-enantiomer products. Despite several reports in the literature describing racemic mixtures of stereoisomers isolated from natural sources, there has not been a comprehensive review of these intriguing racemic natural products. The discovery of many more natural racemates and their potential enzymatic sources in recent years allows us to describe the distribution and chemical diversity of this ‘class of natural products’ to enrich discussions on biosynthesis. In this Review, we describe the chemical classes, occurrence and distribution of pairs of enantiomers in nature and provide insights about recent advances in analytical methods used for their characterization. Special emphasis is on the biosynthesis, including plausible enzymatic and non-enzymatic formation of natural racemates, and their pharmacological significance.

Racemates are equal mixtures of both enantiomers of a chiral molecule. The major chemical building blocks of life, amino acids and sugars, possess a well-defined stereo-configuration making nature a chiral environment<sup>1</sup>. Nevertheless, the occurrence of racemates has become of great importance for science<sup>2,3</sup>. Diverse organisms including animals, plants, bacteria and fungi produce racemates as secondary metabolites (that is, naturally occurring small molecules that are intermediates or products of the metabolism — they are evolutionary diversified and optimized to play important roles in organisms, exhibiting diverse biological activities with applications in medicine) with the ratio of each enantiomer varying between different organs of a single organism, for instance roots, leaves, bark, stem, flowers and seeds of plants, or across different species within the same genus or family<sup>4–6</sup>. As a result, the occurrence of racemates is generally under-appreciated. Most research projects in natural product chemistry focus on isolation, structure elucidation and biological activity evaluation of compounds from natural sources but neglect chiral resolution and absolute configuration determination.

Enantiomers of a molecule share identical physico-chemical properties in an achiral environment, such as organic solvents. Therefore, most chiral secondary metabolites reported to date could have been racemic

or scalemic (enantiomers in unequal amounts) mixtures as only few works have disclosed the enantiomeric ratio of isolated compounds<sup>7</sup>. Batista et al. found that only 11% of works describing chiral metabolites (across 268 research papers published in the *Journal of Natural Products*) contained information about the evaluation of the enantiomeric ratio of isolated compounds<sup>8</sup>. In the classical chemical structure determination workflow, speculations that a compound is a racemate start with the value of its optical rotation; a pure racemic mixture has an optical rotation value of zero. However, the enantiomeric mixture state of a sample can still be verified with an optical rotation value of up to  $\pm 14$  (REF<sup>9</sup>), posing the question of the optical rotation threshold to consider a compound optically pure.

Numerous other questions are encompassed in the topic of natural racemates. Research is ongoing to understand their biosynthetic origins and functions in nature<sup>10,11</sup>. In nature, secondary metabolites do not intervene in the primary metabolism functions of cells essential for growth and development but, rather, improve the survival of organisms (for example, competition, defence, inter-communication) in their natural habitats<sup>12</sup>. Therefore, questions have arisen about the specific reasons for the occurrences of natural product racemates. In addition, this subject requires even greater

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attention as the enantiomers of a molecule rarely exert a synergistic effect towards a pharmacological response, and in some cases pairs of enantiomers display different activities towards a biological system<sup>13</sup>. For example, whereas one component of the pair is highly active and produces beneficial effects, its congener is inactive against the same pathogen or is toxic<sup>13,14</sup>.

This Review focuses on natural products (secondary metabolites) and presents a comprehensive and up-to-date overview of the occurrence, classes and distribution of racemates in nature. Looking at racemates as a ‘class’ of secondary metabolites, important aspects related to their classification, recent advances in analytical developments, biosynthetic discussions and pharmacological relevance are also covered, with extensive referencing of more detailed reviews covering each of these topics when available.

### Racemic natural products

Natural products (secondary metabolites) originate from three main sources: plants, microbiota and animals. Owing to their historical therapeutic significance, natural products have attracted great attention in medicine, chemistry and biology. Research works have focused mainly on higher organisms, such as terrestrial or marine plants and animals, and microorganisms collected from soil, animals and plants. In the frame of this study, we explored 371 research articles (across different journals) describing natural racemates, and found that out of the 778 compounds described, 66% were from plants, 25% from microorganisms and 9% from animals (FIG. 1a,b). This distribution could be explained by the increased number of works on plants compared with other sources. The articles consulted for this comprehensive survey were retrieved from SciFinder<sup>®</sup>, PubMed and Google Scholar when entering the keywords ‘racemic and nature’ or ‘racemic + racemate(s) + natural products’ and refining the search with different concepts/keywords including racemates, compounds, metabolites, nuclear magnetic resonance (NMR), plants, sponges, marines, endophytes, microorganisms, mushrooms, enantiomeric and enantiomers without a limitation of publication date. Some racemates may have been missed in this survey because of the search criteria, references and engines used, but to the best of our knowledge, the largest majority of racemates reported to date from nature has been covered and the present percentages represent the general tendency.

Throughout our investigation, it was not possible to correlate racemate occurrences with plant families, genera or species. This limitation also goes for the other

sources, such as microorganisms and animals, although other reports have suggested that there are classes of natural products in animals, for example dopamine-like compounds, which occur as racemates predominantly<sup>15</sup>. Racemates can be grouped by how they are generated, either by considering their main carbon scaffolds or by exploiting their key racemization processes. Moreover, the biogenetic precursors of certain secondary metabolites (for example, meroterpenes) are more highly reactive towards racemization, including through non-enzymatic processes, and so are more prone to afford racemates (FIG. 1b). As a result, the classification we chose from the collected data is to group racemates based on their main chemical classes (and biosynthetic origin) of their secondary metabolites. FIGURE 2 shows the chemical structures of representative examples of racemates selected from each main chemical class. Supplementary Figure S1 shows the structures and classes of all 778 racemates enumerated from the literature. Alkaloids, polyketides and meroterpenes account for more than half of the natural racemates described in the literature (FIG. 1b).

### Racemic alkaloids (nitrogen-containing compounds).

Racemic alkaloids are a group of racemates with great chemical diversity and various natural origins<sup>16–23</sup> (FIG. 2a). They represent the largest group of naturally occurring racemates encountered to date. Many alkaloids are constituted by indole derivatives or amino acid moieties. The indole-derived alkaloids that have thus far been reported as racemates feature L-tryptophan cores. Racemization occurs either from cyclization, oxidation substitution or dimerization of the amino acid precursor to give, for example, peganumaline A (1). Racemic isoindolinones were also encountered in both plants and ascomycetous fungi. Likewise, alkaloids derived from L-lysine, namely indolizidine, quinolizidine or piperidine-type, yielded racemates<sup>22,24–26</sup>. In the naphtho-indolizidine series, for instance, Al-Khdhairawi et al. reported one of the unique members of benzopyrroloisoquinoline dimerized (that is, constituted by two identical or similar structural moieties) along a cyclobutane ring<sup>25</sup>. Tengerensine (2) is one such rare unsymmetrical cyclobutane possessing two alkaloid moieties, mirror images of one another.

In addition to alkaloids deriving from indoles or amino acids such as those listed above, the nitrogen atom(s) in some alkaloids derived from the introduction of other chemical scaffolds, including anthranilate, pyrimidine and imidazole, or from amination. For example, the racemate oxazin A (3) is a chromene-based polyketide whose nitrogen atom was brought in by an anthranilate derivative followed by subsequent cyclization and dimerization<sup>27</sup>. Likewise, Tian et al. reported nigepladine A (4), a racemate comprising a unique tricyclo[5.4.0.1]dodecane ring system from the dimerization of two thymoquinones bridged by a nitrogen in the Chinese medicinal plant *Nigella glandulifera*<sup>28</sup>. Similarly, bialternacin A (5) is a dimeric polyketide containing a nitrogen probably originating from the amination of one of its biosynthetic precursors<sup>29</sup>. The animal kingdom has produced unique racemic alkaloids, including eudistidine C (6), featuring a tetracyclic core

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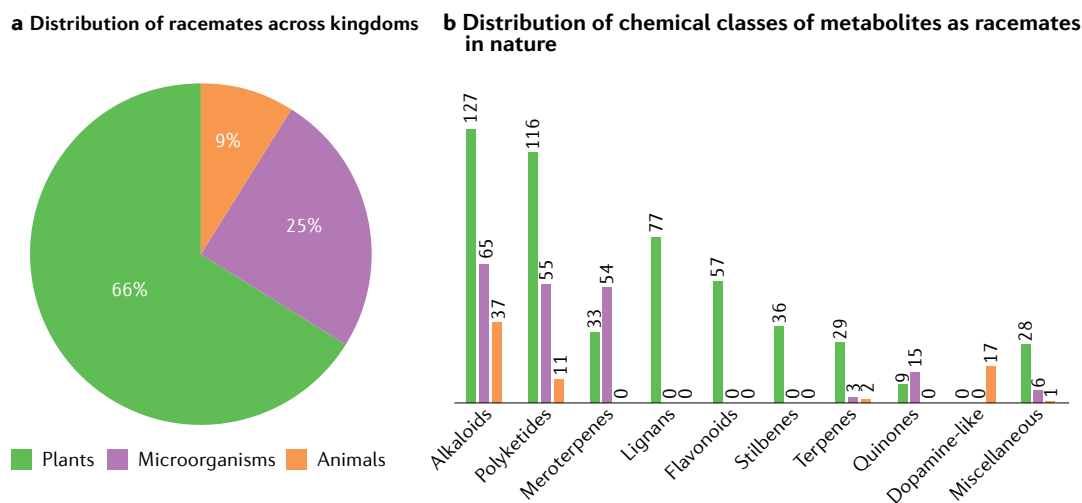


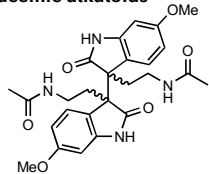
Fig. 1 | **Distribution and diversity of racemates in nature according to up-to-date published data.** Labels of chemical structures are coloured to highlight the sources in nature: green for plants, purple for microorganisms and orange for animals. **a** | Distribution of naturally occurring racemates that have been discovered across different kingdoms: 66% in plants, 25% in microorganisms and 9% in animals. **b** | Distribution of racemic compounds according to their main chemical classes or bio-synthetic origins and across different source kingdoms. Alkaloids = nitrogen-containing compounds including amides; polyketides = keto-methylene chains (cyclic or not) including phloroglucinols; terpenes = mainly linear (or monocyclic) sesquiterpenes but also monoterpenes, diterpenes and sesterterpenes; miscellaneous = compounds belonging to other classes not listed in the row. Supplementary Fig. S1 shows the structures of 778 compounds and 248 reference articles.

elaborated with fused pyrimidine and imidazole rings integrating guanidine and amidine structural groups<sup>30</sup>. A marine sponge of the genus *Suberea* releases the racemate lihouidine (**7**) made up of a benzo[de](1,6)naphthyridine fused with a quinoline-derived system and dimerized through a six-membered unit containing a spiro carbon<sup>31</sup>. Xylaridine A (**8**) is a racemate characterized by a linear fused 5/6/6 tricyclic ring system with another angular fusion of a special 2-azaspiro[4.4]nonane unit<sup>32</sup>. Thiohydantoin derivatives were also reported as racemates, as exemplified by macathiohydantoin H (**9**) from the rhizomes of the Chinese 'maca' plant (*Lepidium meyenii*)<sup>33</sup>. Puralin (**10**), a bromotyrosine-derived spiro-isoxazole-type compound isolated from the marine sponge *Pseudoceratina* sp., is an example of unique spiro-isoxazole derivatives<sup>34</sup>. Further types of racemic alkaloids feature isoxazole, tetramic acid and pyrrolidone moieties<sup>23,35–37</sup>.

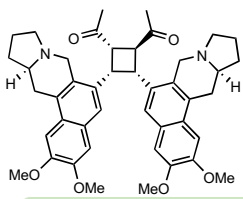
**Racemic polyketides.** Polyketides contain at least two carbonyl groups (or the reduced forms) adjacent to the same carbon atom. Compounds in this category are structurally complex and diverse. Racemization in polyketides occurs mainly during polymerization and subsequent cyclization<sup>38,39</sup>. Oxidized acylphloroglucinols (that is, 1,3,5-trihydroxybenzene derivatives) or polyketides undergo cycloaddition after chain elongation leading to more complex structures<sup>40–44</sup>. Some examples of polyketides include kingianins, which are 4/6/6/6/4 fused pentacyclic rings arising from the dimerization (cycloaddition) of two bicyclo[4.2.0]octa-2,4-diene monomers<sup>45</sup>. The chemical structure of kingianin E (**11**) represents this group of natural products (FIG. 2b). Kingianins constitute one of the largest members of racemic polyketides. Racemic kingianins have been

isolated from the bark of *Endiandra kingiana*. Likewise, sancti analogues, such as sancti C (**12**) in FIG. 2b, feature a dibenzo-2,8-dioxabicyclo[3.3.1]nonane arrangement originating from two chromene precursors<sup>46</sup>. Isolated kingianin analogues mentioned in the literature were dimeric and trimeric derivatives. The occurrence of racemic polyketides bearing a quinone unit is scarce owing to their limited stability. Racemates of tsavoenone C (**13**) and analogues featuring a 1,7-dioxadispiro[4.0.4.4]tetradecane were isolated from the lichen *Parmotrema tsavoense*<sup>47</sup>. The other racemic quinone analogues were derived from naphthoquinone<sup>48,49</sup>.

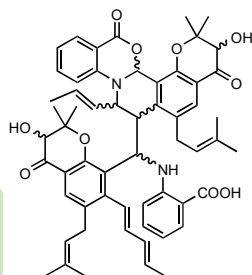
**Racemic meroterpenes.** Meroterpenes have a mixed biosynthetic origin (terpenoid/polyketide) as they are produced after the condensation of a terpene (sesquiterpene or monoterpene) with a phenolic compound (quinone, phloroglucinol, guttiferone and so on)<sup>50–52</sup>. Meroterpenes from the *Hypericum*, *Dryopteris* and *Psidium* genera feature  $\alpha$ -humulene fused through its C-6/C-7 double bond to a *cis*-oriented  $\alpha,\beta$ -unsaturated ketone of acylphloroglucinols, for example drychamponone A (**14**)<sup>53–55</sup> (FIG. 2c). Other members of this group include a spiro system from the C-7/C-13 double bond of  $\beta$ -humulene and an oxidated acylphloroglucinol<sup>56</sup>. Hypulatone A (**15**), isolated from *Hypericum patulum*, features a spiro[benzofuran-2,1'-cycloundecan]-4'-ene-4,6(5H)-dione moiety and represents a key example of meroterpenes bearing a spiro system<sup>56</sup>. Another series in this group consists of rearranged prenylated hydroquinone or resorcinol leading to various ring closure alternatives including 6/6/6/4, 6/6/5/4 and 6/6/5/5 rearranged systems, and others<sup>51,57</sup>. The latter class of racemic meroterpenoids were isolated from the fruiting bodies of wild fungi belonging to the *Ganoderma* genus<sup>58–61</sup>.

**a Racemic alkaloids**


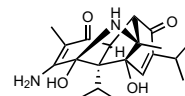
(±)-1  
peganumaline A  
Source: *Peganum harmala*



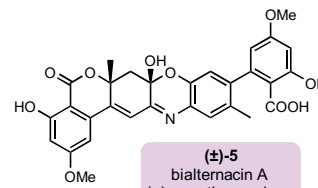
(±)-2  
tengerensine  
(+)-enantiomer given  
Source: *Ficus fistulosa*  
var. *tengerensis*



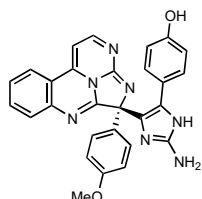
(±)-3  
oxazinin A  
Source: marine fungus  
*Eurotiumycetes* strain 110162



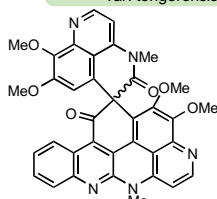
(±)-4  
nigegladiene A  
(-)-enantiomer given  
Source: *Nigella glandulifera*



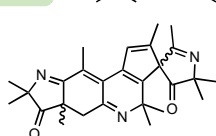
(±)-5  
bialternacin A  
(+)-enantiomer given  
Source: *endophytic*  
*Alternaria* sp.



(±)-6  
eudistidine C  
(-)-enantiomer given  
Source: *Eudistoma* sp.

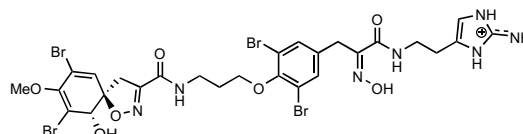
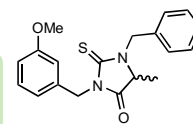


(±)-7  
lihoididine  
Source: genus *Suberea*

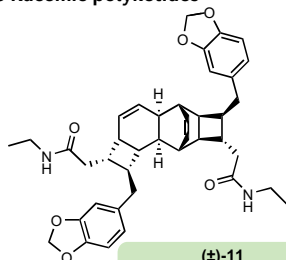


(±)-8  
xylaridine A  
Source: *Xylaria longipes*

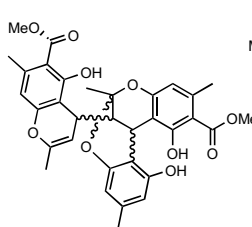
(±)-9  
macathiohydantoin H  
Source: *Lepidium meyenii*  
Chinese 'maca' plant



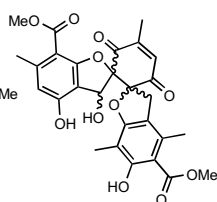
(±)-10  
purealin  
(-)-enantiomer given  
Source: *Pseudoceratina*  
marine sponge

**b Racemic polyketides**


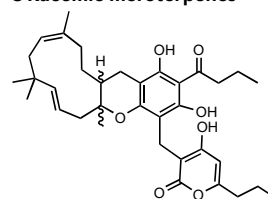
(±)-11  
kingianin E  
(-)-enantiomer given  
Source: *Endiandra kingiana*



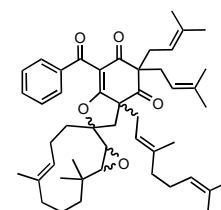
(±)-12  
sancti C  
Source: *Parmotrema*  
*sancti-angelii* lichen



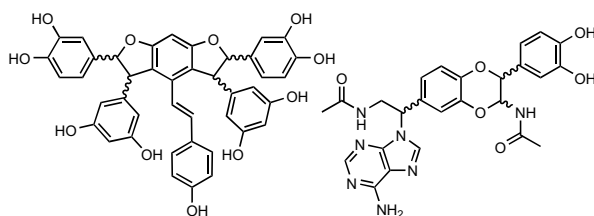
(±)-13  
tsavoene C  
Source: *Parmotrema*  
*tsavoense*

**c Racemic meroterpenes**


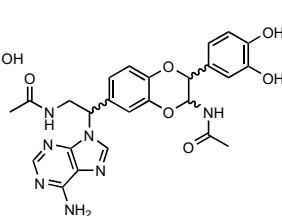
(±)-14  
drychampone A  
Source: *Dryopteris championii*



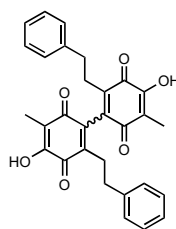
(±)-15  
hypulatone A  
Source: *Hypericum patulum*

**d Phenylpropanoid-condensed racemates**


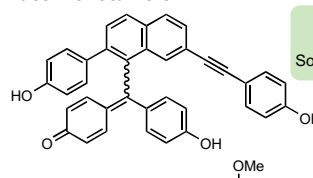
(±)-16  
(*E*)-mesocyperusphenol A  
Source: *Cyperus rotundus*



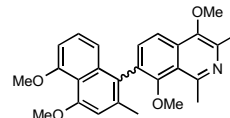
(±)-17  
aspongopamine B  
Source: *Aspongopus chinensis*  
insect



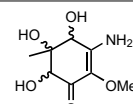
(±)-18  
parvistemin A  
Source: *Stemona parviflora*

**e Racemic rotamers**


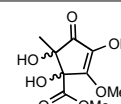
(±)-19  
selaginellin A  
Source: *Selaginella tamariscina*



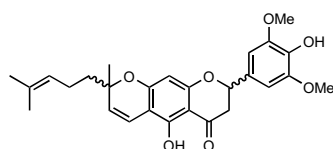
(±)-20  
(*S*)-dioncophyllacine A  
Source: *Triphyophyllum peltatum*



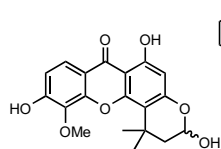
(±)-24  
cyclohexenone class  
Source: *Alternaria* sp.



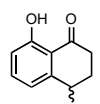
(±)-25  
cyclopentenone class  
Source: *Alternaria* sp.

**f Other phenolics and miscellaneous**


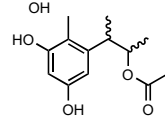
(±)-21  
paulownione C  
Source: *Paulownia tomentosa*



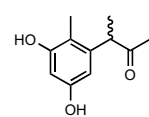
(±)-22  
cratosumatranone D  
Source: *Cratogeomys sumatranum*



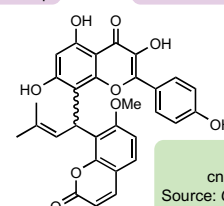
(±)-23  
regiolone  
Source: *Juglans regia*



(±)-26  
penicillide A  
Source: *Penicillium* sp.



(±)-27  
penicillide B  
Source: *Penicillium* sp.



(±)-28  
cnidimonin A  
Source: *Cnidium monnieri*

**Phenylalkenoid-condensed racemates.** Compounds in this family are made up of two or more units of phenolic compounds linked to one another due to various oxidation processes, among which cycloaddition is the most common reaction<sup>62–65</sup>. Racemization in stilbenes,

such as (*E*)-mesocyperusphenol A (16), is observed in dimeric and trimeric molecules<sup>64</sup> (FIG. 2*g*). Condensation of the *ortho*-positioned hydroxyls in the catechol moiety of stilbenes affords more structurally complex racemic natural products<sup>66</sup>. The same is true for the

◀ Fig. 2 | **Main groups and examples of natural racemates.** Labels of chemical structures are coloured to highlight the sources in nature: green for plants, purple for microorganisms and orange for animals. **a** | Racemic alkaloids peganumaline A (**1**)<sup>174</sup>, tengerensine (**2**)<sup>25</sup>, oxazinin A (**3**)<sup>27</sup>, nigebladine A (**4**)<sup>28</sup>, bialternacin A (**5**)<sup>29</sup>, eudistidine C (**6**)<sup>30</sup>, lihouidine (**7**)<sup>31</sup>, xylaridine A (**8**)<sup>32</sup>, macathiohydantoin H (**9**)<sup>33</sup> and purealin (**10**)<sup>34</sup>. **b** | Racemic polyketides kingianin E (**11**)<sup>175</sup>, sancti C (**12**)<sup>146</sup> and tsaoenone C (**13**)<sup>147</sup>. **c** | Racemic meroterpenes drychampone A (**14**)<sup>54</sup> and hypulatone A (**15**)<sup>56</sup>. **d** | Phenylpropanoid-condensed racemates (*E*)-mesocyperusphenol A (**16**)<sup>56</sup> and aspongopamine B (**17**)<sup>70</sup>. **e** | Racemic rotamers parvistemin A (**18**)<sup>76</sup>, selaginellin A (**19**)<sup>176</sup> and (*S*)-dioncophyllacine A (**20**)<sup>78</sup>. **f** | Other phenolics and miscellaneous paulownione C (**21**)<sup>177</sup>, cratosumatranone D (**22**)<sup>153</sup>, regiolone (**23**)<sup>178</sup>, (4*R*\*,5*S*\*,6*S*\*)-3-amino-4,5,6-trihydroxy-2-methoxy-5-methyl-2-cyclohexen-1-one (**24**)<sup>82</sup>, (4*S*\*,5*S*\*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten-1-one (**25**)<sup>82</sup>, penicilliode A (**26**)<sup>84</sup>, penicilliode B (**27**)<sup>84</sup> and cnidimonin A (**28**)<sup>81</sup>.

1,2,3-trihydroxydiphenoxylpropanoids group which is mostly found in plants. Upon their C–C or C–O cycloaddition, condensation occurs to generate phenolic compounds which are racemates<sup>67–70</sup>. Similar behaviours are observed in the formation of some lignan-type racemates<sup>71–74</sup>. Phenylalkenoid-condensed racemates constitute the most prevalent group of racemates in the animal kingdom, especially in ants through acetylated dopamine dimer analogues, including aspongopamine B (**17**). Indeed, dopamine undergoes dimerization to induce dioxane ring closure, from the reaction involving constituent ethylene side chain and catechol hydroxyls<sup>15,65,70</sup>. In nature, every molecule of the latter group, dopamine-like natural products, exists as a racemate<sup>15</sup>. Phenylpropanoids are involved in the racemization of some indoles and dihydrobenzophenanthridines, xanthenes and coumarins<sup>67–69,75</sup>.

**Racemic rotamers.** Three main scaffolds of racemates exhibiting an axis of chirality have been found in the literature. Atropisomers arise when the rotation barrier energy along the  $\sigma$ -stretching bond is high, preventing interconversion of optically active stereoisomers and leading to the isolation of enantiomers for molecules in which that axis is the only stereogenic element; otherwise, diastereomers might be produced. Many factors can cause racemization to occur, including a low steric hindrance and tautomerisms. For instance, the racemization of phenylethylbenzoquinones, such as parvistemins (for example, parvistemin A (**18**)<sup>76</sup>), is caused by the lack of steric hindrance and biosynthetic origin. These structures are generated from dimerized and oxidized hydroxylbenes. Selaginellins, the major chemical constituents of the *Selaginella* genus and naturally occurring pigments from plants, contain a *p*-quinone methide (QM) and alkynylphenol moieties as shown in the chemical structure of selaginellin A (**19**)<sup>77</sup> (FIG. 2e). Both QM and phenol groups of one side of the  $\sigma$ -bond undergo keto–enol tautomerism, meaning the compounds exist as a mixture of non-separable enantiomers. Alkylation of the phenol fragment prevents the tautomerization occurring but racemization continues from free rotation along the  $\sigma$ -bond axis. Some isolated racemates from *Selaginella pulvinata* are ethoxyl derivatives of common selaginellins (with free phenol groups and where tautomerization occurs) in which racemization was from the combined actions of axial chirality and molecular rotation. Moreover, a racemic alkaloid called dioncophyllacine A (**20**), from the leaves of *Triphyophyllum peltatum*,

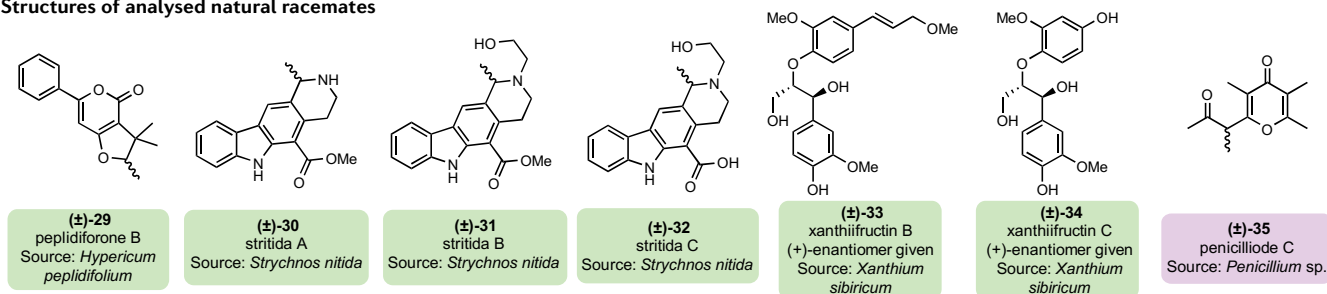
exhibits axial chirality from the coupling of a naphthalene and an isoquinoline moiety. Racemization occurs because the allowed rotation along the chiral axis causes the formation of equal amounts of both enantiomers of the chiral molecule<sup>78</sup>.

**Other phenolics and miscellaneous.** Common secondary metabolites such as flavonoids (for example, paulownione C (**21**)<sup>79,80</sup>, benzophenones/xanthenes (for example, cratosumatranone D (**22**)<sup>52</sup>, chromenes (for example, regiolone (**23**)<sup>81</sup>, cycloalkenes (for example, **24**, **25**)<sup>82</sup> and phenolic acids (for example, penicilliodes A and B (**26** and **27**)<sup>83</sup> also occurred as racemates without any oxidative cyclization as is typical for the aforementioned racemates<sup>80,84</sup> (FIG. 2f). Several compounds in this group were elaborated from either inter-species or intraspecies coupling or following oxidation–cyclization patterns of their substituents (cnidimonin A (**28**)). Some racemates in this group have a single stereocentre (that is, less complexity) as their core skeletons are benzene and analogues. Racemates with more than one asymmetric carbon arise mainly when the key basic scaffold is from terpenes or cyclohexenes<sup>9,85–87</sup>. The biosynthesis of such compounds could be of great interest.

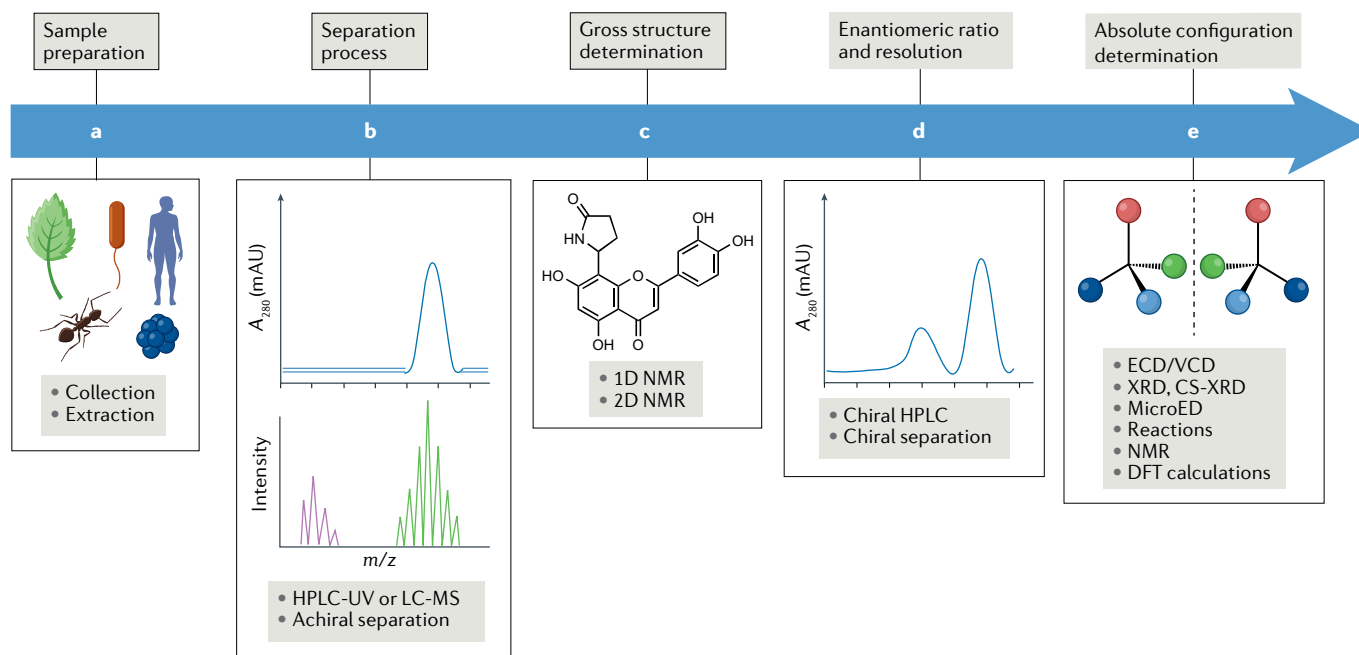
### Advanced analysis methods

The recognition of a racemic mixture starts with optical rotation determination. In general, optical rotation values of racemates should be close to zero or equal to zero (that is, in cases where the racemate does not contain any impurities). However, racemates containing some minor optically active impurities have been found to have an optical rotation value of up to  $\pm 14$  (REF.<sup>9</sup>). Therefore, some racemates with non-zero optical rotation values have been described as enantiopure compounds in the literature when only optical rotation was used without combining it with other analytical methods. As such, emphasis should be placed on the enantiomeric ratio evaluation of chiral compounds, even for those with higher optical rotation values. Electronic and vibrational circular dichroism (ECD and VCD) are oftentimes combined with optical rotation data to identify enantio-enriched mixtures<sup>88,89</sup>. Peplidiforone B (**29**) (FIG. 3A), isolated from *Hypericum peplidifolium*, was confirmed to be a racemate as it exhibited no Cotton effect, the characteristic change in the absorption of a circularly polarized light around the absorption band of a chiral compound, in its ECD spectrum along with an optical rotation value close to zero<sup>90</sup>. Besides chiroptical methods, well-elaborated chromatographic techniques for racemate recognition (enantiomeric ratio determination), separation and quantification techniques also exist nowadays. Chiral nanoparticles and chiral nanographene-based column materials for high performance liquid chromatography (HPLC), gas chromatography, capillary electrophoresis and capillary electrochromatography have been recently reviewed<sup>91,92</sup>. In natural products or pharmaceutical research, the separation and quantification of pairs of enantiomers are almost always established on chiral HPLC, gas chromatography or supercritical fluid chromatography (SFC), from which numerous methods have been developed and reviewed<sup>8,93–95</sup>. The best of them

## A Structures of analysed natural racemates



## B Analytical techniques



**Fig. 3 | Analysis approaches for natural racemates.** Labels of chemical structures are coloured to highlight the sources in nature: green for plants and purple for microorganisms. **A** | Structures of analysed racemates peplidiforone B (**29**)<sup>179</sup>, stritidas A–C (**30–32**)<sup>20</sup>, xanthiifructin B (**33**)<sup>97</sup>, xanthiifructin C (**34**)<sup>97</sup> and penicillide C (**35**)<sup>44</sup>. **B** | Analysis approaches for natural racemates, from extraction and separation to structure elucidation with absolute configuration determination. Detailed reviews on these techniques are published elsewhere<sup>67,79,99,103</sup>. **Ba** | Extraction/preparation: during dryness, sun light/energy and high temperature (>24 °C) could induce transformations including racemization. Uses of liquid nitrogen should avoid such transformations leading to artefacts, allowing extraction of genuine natural racemates. **Bb** | Separation process: basic chromatographic methods such as column chromatography and thin layer chromatography can afford unnatural racemic mixtures (artefacts) from complex extracts. High performance liquid chromatography (HPLC), online liquid chromatography and liquid chromatography–mass spectrometry

(LC-MS) improve the isolation avoiding possible racemization due to silica–sample or inter-sample interactions. **Bc** | Structure determination: the gross structure of a racemate could be achieved by means of nuclear magnetic resonance (NMR), mass spectrometry and LC-MS/tandem, HPLC coupled with ultraviolet photodiode array (HPLC-UV), infrared, nuclear Overhauser effect spectroscopy/rotating frame Overhauser effect spectroscopy and X-ray diffraction (XRD) providing required data to define the relative configuration. **Bd** | Enantiomeric ratio determination/resolution: the resolution of racemates could be achieved by various techniques, including chiral HPLC (TABLE 1). **Be** | Absolute configuration determination: several methods were commonly used including electronic circular dichroism (ECD) and vibrational circular dichroism (VCD), experimental and calculated spectra using density functional theory (DFT), Mosher's method (chemical reaction), NMR (<sup>1</sup>H, <sup>13</sup>C), quantum mechanics (for example, DP4+ method), X-ray diffraction, microcrystal electron diffraction (MicroED) and crystalline sponge X-ray diffraction (CS-XRD).

is the so-called 'Inverted Chirality Columns Approach' consisting of the inversion of the elution observed as a response to the change in the chirality of the column — both enantiomeric forms of stationary phases are used in the same column<sup>96</sup>. TABLE 1 summarizes the advantages of analytical techniques used for recognition, resolution and quantification of both enantiomers of a chiral compound in natural product research.

In most cases, chiral HPLC is the preferred method for the resolution of mixtures of enantiomers. However, in

racemic mixtures, some component enantiomers have been reported as non-separable on chiral HPLC. They are exemplified by selaginellin enantiomers, which can be interconverted in solution<sup>77</sup>. Chiral resolution of the constituents of the pyridocarbazole alkaloids, stritidas A–C (**30–32**), failed on a Phenomenex Lux cellulose-2 column<sup>20</sup>. The phenylpropanoid derivatives, xanthiifructins A/B (enantiomeric forms of **33**), were separable on a Chiralpak AD-H column and a Phenomenex Lux cellulose-2 column, but not their analogue xanthiifructin C (**34**)

Table 1 | Advantages and applications of analytical methods used for natural racemates

Method/category	Resolution	Identification	Quantification	Absolute configuration	
HPLC	HPLC-UV <sup>90,95</sup>	Enantiomers are separated on a chiral column	Profiles comparison with standards	Quantification and enantiomeric ratio evaluation of ultraviolet absorbing compounds	Not applicable for new compounds; known compounds required chiral HPLC-UV and reference standards for retention time comparison
	HPLC-ORD <sup>60</sup>	Both enantiomers of the mixtures will present oppositely charged specific rotation when separated on a chiral column	Identifies racemate through the evaluation of optical rotation	Chiral columns also help evaluate the yield of each component of the polar mixture	The methods could help absolute configuration determination especially for compounds with standards or fewer stereocentres (one or two) or P/M chiral axis, but will be limited for more complex scaffolds
	HPLC-CD <sup>88,89</sup>	Can resolve separable enantiomers on a chiral phase	HPLC profiles comparison with known references	Feasible with chiral column HPLC and the method is more accurate as circular dichroism is being used as a detector	The circular dichroism detector helps afford the absolute configuration for compounds which fit into circular dichroism empirical rules or with experimental circular dichroism data of related structures; otherwise, calculated circular dichroism spectra are needed
	HPLC/LC-MS <sup>8,35,92–96</sup>	Enantiomers are separated on a chiral column	Identification with configurationally known standards	Feasible with chiral column HPLC	Marfey method turns enantiomeric mixture of amino acids in diastereoisomers with the chiral Na-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA); absolute configuration is then deduced by online LC-ESI-MS matching with standards
SCFC	SCFC-CS-XRD <sup>94</sup>	Combine advantages of SCFC to work on thermo-labile compounds and those of CS-XRD to apply on liquid and oil samples	Crystal of racemates formed belongs to specific group space leading to their identification	Feasible with SCFC with lower solvent consumption and shorter time than HPLC	CS-XRD makes it possible to solve the absolute configuration of both enantiomers
Gas chromatography	GC-MS <sup>161,162</sup>	Enantiomers are separated over a chiral column but the conditions inherent to gas chromatography methods remain	Using chiral column, identification can be made possible by profile comparison with standards	Indicated for low molecular weight and non-heat-sensitive compounds	Chiral GC-MS analysis enables online comparison of retention times of each enantiomer of a chiral substance with reference standards
XRD	SC-XRD <sup>163</sup>		Racemates crystallize in specific group space but are limited to crystalline solid		Non-centrosymmetric crystal displays the so-called anomalous dispersion effect which breaks down the Friedel law; thus, the absolute configuration could be determined using either the Flack parameter or by comparing the intensities of Bijvoet or Friedel pairs
	CS-SC-XRD <sup>8,112</sup>	Each enantiomer of the racemate — solid, oil or liquid — crystallizes in a unique order, allowing their resolution	As in a classical XRD experiment, the nature of the crystal could be assessed		The method is applied with a metal–organic framework (the host) claiming a specific optical activity; thus, leading to the absolute configuration determination of each enantiomer of the racemate
MicroED <sup>110,111</sup>		This method was used for small molecules only recently and has not yet been coupled to chromatography	Can be an alternative to XRD when crystals of suitable quality are not available for gross structure identification	Applicability in the quantification of natural products still has to be demonstrated	Diffraction-based method similar to XRD but does not have associated limitations; crystals of small sizes and even powders not useful for XRD were analysed by MicroED

Table 1 (cont.) | Advantages and applications of analytical methods used for natural racemates

Method/category	Resolution	Identification	Quantification	Absolute configuration
NMR <sup>9,113</sup>	Affordable when hyphenated to a chiral column HPLC system	Best method to assess the gross chemical structure of a racemate; still, the racemic status of a sample is only displayed under optical active species influence during NMR experiments	Affordable when hyphenated to a chiral column HPLC system or after derivatization	Theoretical assessment of the shielding tensor of various stereoisomers of a compound paired with the high compatibility of calculated and analytical results can lead to absolute configuration determination of stereochemical compounds, in large, and could be applied to a racemate when the NMR is recorded in an optical active solvent
Derivatization <sup>114,115</sup>	Enantiomers are turned to diastereomers and separated on an achiral HPLC system	Enantiomers are turned to diastereomers and spotted on analytical thin layer chromatography	Feasible on an achiral HPLC system after derivatization	Enantiomers could be turned to esters applying Mosher's method, for instance, and analysed by NMR, but it is limited to some classes of compounds

CS-XRD, crystalline sponge X-ray diffraction; GC-MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; HPLC-CD, high performance liquid chromatography–circular dichroism; HPLC-UV, high performance liquid chromatography–ultraviolet photodiode array; LC-ESI-MS, liquid chromatography–electrospray ionization–mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; MicroED, microcrystal electron diffraction; NMR, nuclear magnetic resonance; ORD, optical rotation dispersion; P/M chiral axis, plus (P or Ra or right-handed)/minus (M or Sa or left-handed) stereo-descriptors; SCFC, supercritical fluid chromatography; SC-XRD, single crystal X-ray diffraction; XRD, X-ray diffraction.

which structurally differs by the lack of the propenyl moiety on its second phenyl group<sup>97</sup>. Similarly, the enantiomers of the phenylpropanoid derivative penicillide A (**26**) were not distinguishable on many chiral columns (Phenomenex Lux celluloses-1, 2, 3, 4 and 5 and FLM Chiral ND(2)), whereas their analogues penicillide B (**27**) and penicillide C (**35**) were successfully resolved in their respective enantiomers<sup>44</sup>. In all, available chiral HPLC columns still fail to distinguish between physical properties of components of certain enantiomer mixtures. Alternatively, mixtures of enantiomers can also be resolved by exploiting the difference in the metabolism of both enantiomers by fungi or bacteria, which may recognize only one of the components. Parmaki et al. developed this method for the resolution of racemic mixtures of natural products<sup>98</sup>. The resolution of natural racemates using chiral reagents has not been mentioned in the natural products literature recently, yet this chromatography method is useful for trace amounts of natural racemates<sup>99</sup>. The application of chiral mobile phase additives such as cyclodextrins for the resolution of natural racemic mixtures has not been reported in the literature recently. The occurrence of natural products in relatively low quantity renders difficult the use of derivatization and other reaction-mediated separation of pairs of enantiomers. Another resolution approach not found in the literature on natural product enantiomer separation is the mechanical resolution method, based on differentiation of the salts produced from the acid/base reactions of enantiomers and a crystallizing agent<sup>99</sup>. Enantiomers may not crystallize under the same conditions. Pasteur used this strategy when separating the stereoisomer components of the tartaric acid racemate<sup>100</sup> for the first time. Further alternatives to HPLC include the membrane-based chromatography not yet applied to natural occurring racemates but which may be both effective and sustainable<sup>99</sup>. The quantification of racemates could be achieved by NMR (using chiral solvent and derivatizing agents), ECD, VCD or hyphenated techniques such as high performance liquid chromatography coupled with ultraviolet photodiode

array (HPLC-UV), high performance liquid chromatography coupled with circular dichroism (HPLC-CD) and liquid chromatography coupled with mass spectrometry (LC-MS) on chiral columns<sup>8,101</sup>. TABLE 1 highlights the advantages of each method.

#### Absolute configuration determination

After the recognition and resolution of natural racemic mixtures, the gross structure determination by NMR spectroscopy and mass spectrometry is carried out. These are the most common techniques applied in the structure determination of natural products<sup>102</sup>. FIGURE 3B summarizes the most common analytical approaches from purification to relative and absolute configuration determination.

Classical NMR and mass spectrometry experiments cannot distinguish between pairs of enantiomers in their mixtures<sup>67,103</sup>. Both having the same physical properties, only one set of signals are observed in the NMR spectra of a racemic mixture. The presence of two or more sets of NMR signals is a sign of a mixture of either non-related compounds or diastereomers in the sample<sup>36,104</sup>. Besides NMR, X-ray diffraction (XRD) is also currently used to identify components of a racemic mixture. Diffraction data of the crystalline form of the sample mixture are obtained under a certain light<sup>105</sup>, most likely under CuK $\alpha$  conditions in conjunction with Friedel values and the Flack parameter of the compound<sup>106,107</sup>. The key drawbacks of this technique are the difficulty in the crystallization of some sample mixtures and its limitation to solid samples. Recently, the cryogenic electron microscopy method known as microcrystal electron diffraction (MicroED)<sup>108–111</sup> was developed as a very promising alternative to XRD, achieving structure elucidation of natural products using crystals of small sizes and poor quality or powders not suitable for XRD. However, its broad use and applicability in the field of natural products has yet to be demonstrated<sup>111</sup>.

XRD and Fourier transform XRD (FT-XRD or fast FT-XRD), especially in their current modern states, are



useful tools for determining the absolute configuration of crystalline and enantiomeric natural products<sup>8</sup>. Modern workflows combine XRD, circular dichroism calculations and, sometimes, optical rotation dispersion (ORD) spectroscopy. A recent XRD technique proposed the use of metal–organic frameworks to host the mixture of enantiomers and the resultant single crystal of the hybrid material is examined by XRD<sup>112</sup>. As a result, enantiomers within the mixture adopt a certain order in the porous hole of the metal–organic framework leading to a clear absolute configuration determination. The method is called crystalline sponge XRD (CS-XRD) and has proven to help in the structure elucidation of samples such as oils, liquids or other non-crystalline solids<sup>112</sup>.

Both XRD and circular dichroism are powerful techniques for absolute configuration determination of chiral molecules. Regardless of the method applied, the calculated circular dichroism spectra of each of the stereoisomers of the gross structure determined from NMR are assessed by density functional theory (DFT) calculations. Absolute configurations are assigned to each of the enantiomers by matching these spectra to the experimental ones. When the gross structure contains more than two stereocentres, the initial XRD structures could help eliminate some stereoisomers prior to the DFT calculations. Where neither VCD nor ECD methods are applicable, NMR coupled to quantum mechanics can still serve to assess the absolute configuration of a compound. Here, enantiomers are derivatized with a chiral reagent, separated into the corresponding diastereomers and their 1D NMR data recorded. Then, conformers of the gross structures are geometry-optimized using density functional methods, for instance B3P86/6-31G(d), B3LYP/6-31G(d), B3LYP/6-311++G(2d,p) and MPW1PW91/6-31G(d,p), and sorted according to the nuclear Overhauser effect data recorded<sup>67,103,113</sup>. The <sup>13</sup>C and <sup>1</sup>H NMR shielding tensors of each stereoisomer of retained diastereomers are simulated using quantum mechanics. The DP4+ probability method<sup>114</sup> is the best approach to achieve this. However, the method becomes increasingly complex as the number of stereogenic carbons rises.

Chiral HPLC and gas chromatography analyses<sup>35</sup> are also absolute configuration determination strategies. Configurations of enantiomers can be achieved by online comparison of retention times of each enantiomer with those of chiral references. The Marfey method, for instance, can be applied to online liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) to identify the absolute configuration of amino acids using the derivatizing agent *N*α-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA)<sup>35</sup>. Synthetic methods have also been developed to elucidate the absolute configuration of compounds. The most popular of which is the Mosher method for secondary alcohol or amine-bearing stereocentres. The technique assesses the anisotropic effects of the phenyl group of a derivatizing chiral agent, α-methoxy-α-trifluoromethylphenylacetic acid (MTPA), in the <sup>1</sup>H NMR of the esterified racemate hybrids<sup>115</sup>. Moreover, secondary alcohol-containing enantiomers undergo acylation by lipases, such as from *Candida* or

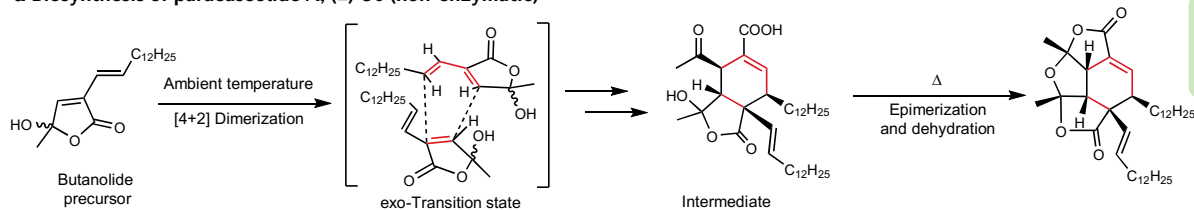
*Pseudomonas* spp. or cholesterol esterase, under catalysis conditions with distinct kinetics. Under the same conditions, the *R*-enantiomer of the racemate is most likely resolved faster than the *S*-congener from the hydrolysis of the ester or acylation of the alcohols, following Kazlauskas' empirical rules<sup>116</sup>.

### Biosynthetic basis of racemization

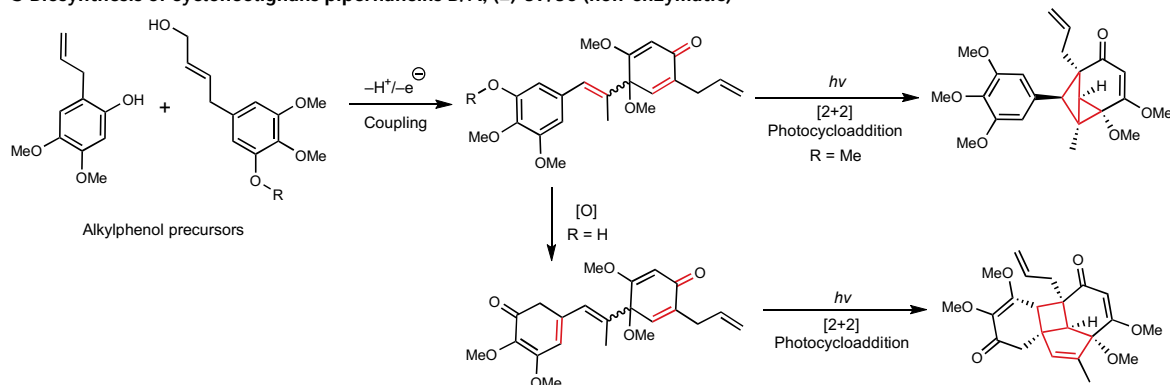
The biosynthetic basis of racemization has always intrigued scientists. Most racemates are not a product of a clear enzymatic pathway but, rather, are considered by-products of secondary metabolism. In general, racemization of a given chiral molecule might be a result of its physical properties (for example, solubility) and chemical stability (for example, due to pH or temperature changes)<sup>117,118</sup>. Racemates could also be intermediates towards the formation of scalemic mixtures or enantiopure compounds. The excellent review by Ballard et al. provides detailed mechanistic and kinetic insights into the racemization and enantiomerization (conversion of one enantiomer into the other) of selected drugs by chemical processes in aqueous solutions (for example, pH variations) and the vapour phase (for example, pyrolysis), or racemization in plasma and serum albumin solutions<sup>117</sup>. Our focus in this section is natural products produced by living (micro)organisms as racemates, as opposed to artefacts arising from extraction or experimental procedures.

Some plausible mechanisms for the formation of racemic natural products are depicted in FIG. 4. Reactions governing the formation of some groups of racemates in nature are subject to exogenous factors (for example, temperature or light) or high reactivity of biogenic intermediates which can undergo spontaneous non-enzymatic transformations. For instance, increased temperature can trigger a cascade of reactions with successive oxidation, elimination and cyclization transformations yielding new racemic molecular scaffolds<sup>119</sup>. These non-enzymatic racemization processes resulting in new racemates are exemplified by the proposed biosynthesis of the structurally unique natural product paracaseolide A (**36**), which is a racemate hypothetically formed through a spontaneous Diels–Alder [4 + 2] cycloaddition (enzyme-free dimerization) starting from two α-dodecenylobutenolide molecules, followed by the epimerization and dehydration of the resulting Diels–Alder product<sup>120</sup> (FIG. 4a). Another example features the neolignans piperhancins (**37**, **38**), whose plausible biogenesis involves the oxidative coupling of alkylphenol derivatives and subsequent intramolecular [2 + 2] photocycloaddition<sup>74</sup> (FIG. 4b). Biomimetic synthesis as a tool to validate the biosynthetic formation of racemates through non-enzymatic (chemical) racemization has been intensively discussed in the literature and key examples are summarized in TABLE 2. Still, much is unknown in the biosynthesis of the majority of racemates, as they are not clearly explained by non-enzymatic mechanisms, such as non-stereospecific ring closure or cycloaddition.

Enzymes are the metabolism engines of every biological system. They are homochiral ensembles, possessing uniform handedness, and control the carbon framework of each organism cell<sup>121</sup>. It is quite common for amino

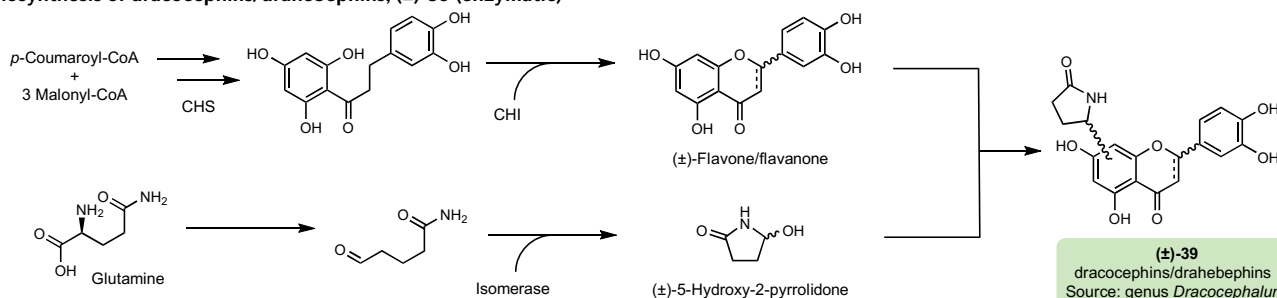
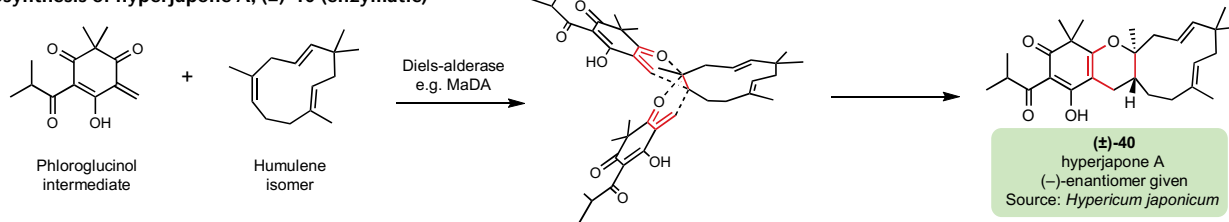
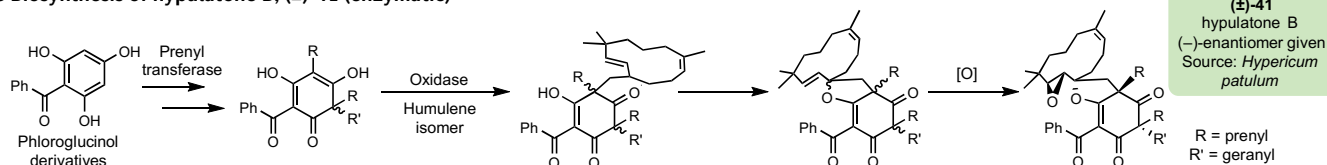
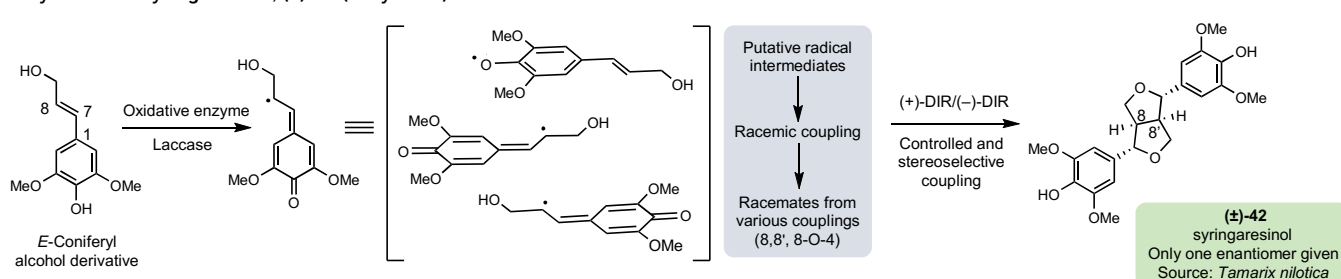
**a Biosynthesis of paracaseolide A, (±)-36 (non-enzymatic)**


(±)-36  
paracaseolide A  
One enantiomer given  
Source: *Sonneratia paracaseolaris*

**b Biosynthesis of cycloneolignans piperhancins B/A, (±)-37/38 (non-enzymatic)**


(±)-37  
piperhancin B  
(+)-enantiomer given  
Source: *Piper hancei*

(±)-38  
piperhancin A  
(+)-enantiomer given  
Source: *Piper hancei*

**c Biosynthesis of dracocephins/drahecephins, (±)-39 (enzymatic)**

**d Biosynthesis of hyperjapone A, (±)-40 (enzymatic)**

**e Biosynthesis of hypulatone B, (±)-41 (enzymatic)**

**f Biosynthesis of syringaresinol, (±)-42 (enzymatic)**


◀ Fig. 4 | **Proposed biosynthetic basis to some racemates.** Labels of chemical structures are coloured to highlight the sources in nature: green for plants. **a** | Hypothetical biosynthesis of (±)-paracaseolide A (**36**) proposed by Wang and Hoyer<sup>120</sup> through biomimetic synthesis. A key step is the non-enzymatic and spontaneous [4 + 2] Diels–Alder dimerization (cycloaddition) of the butanolide precursor at ambient temperature. **b** | Proposed biosynthesis of cycloneolignans (±)-piperhancins B/A (**37/38**) by Yang et al.<sup>74</sup>. Alkylphenol precursors undergo a radical coupling to produce neolignan intermediates. The key non-enzymatic event features the intramolecular [2 + 2] photocycloaddition of the cycloenone with a double bond (red) in both intermediates to yield **37** and **38**. **c** | Biosynthetic basis to racemic pyrrolidinone-containing flavonoids (**39**) as proposed by Wang et al.<sup>180</sup>. This bioprocess involves chalcone synthases (CHS) and epimerases as bioengineers. Epimerases such as chalcone isomerases (CHI) have been characterized in the conversion of chalcone to racemic flavanones/flavones. It could also be hypothesized that considering the fermentation process of Wang et al. to produce pyrrolidinone catechin derivatives, an epimerase likely catalyses the cyclization of 4-oxobutanamide to pyrrolidinone. The condensation of flavanone/flavone and pyrrolidinone intermediates afford **39**. **d** | Hypothetical intervention of Diels-alderases in cycloaddition. Intermolecular Diels-alderases similar to the recently discovered *Morus alba* Diels-alderase (MaDA) could catalyse the formation of racemic natural products such as **40** through [4 + 2] cycloaddition<sup>55,128</sup>. **e** | Proposed oxidative coupling to (±)-hypulatone B (**41**)<sup>56</sup>. The terpene and phloroglucinol derivatives undergo an enzymatic oxidative coupling (catalysed by an oxidase) followed by subsequent cyclization and oxidation to give **41**. **f** | Proposed enzymatic biosynthesis of racemic lignans in the presence or absence of dirigent proteins (DIRs). Hypothetical intervention of two DIRs of opposite stereoselectivities could yield racemate (±)-syringaresinol (**42**) preferentially with regioselectivity and stereoselectivity<sup>131,133,181</sup>.

acids and resulting derivatives, proteins, peptides and, to some extent, enzymes to exist under the L-stereoisomer in nature<sup>122</sup>. Interestingly, D-amino acids, such as alanine and serine, were found to occur as components of peptidoglycans, teichoic acids and poly-γ-glutamate capsules in bacteria<sup>122</sup>. D-Isomeric amino acids have also been found in other living organisms including plants and animals<sup>122</sup>. D-isomers are far less abundant in nature than L-isomers and may also result from the isomerization of the latter in the living habitat. Enzymes such as racemases and epimerases are responsible for this interconversion of stereochemistry at a given chiral centre<sup>122</sup>.

In this regard, the occurrence of racemates becomes clear to a certain extent; enantio-enriched species could undergo racemization under the influence of enzymes. Essentially, racemases and epimerases, such as alanine racemase<sup>122</sup> and cellobiose 2-epimerase<sup>123</sup>, catalyse the cleavage and reformation of a bond around a stereocentre, accompanied by an epimerization of the site. This conflicts the consensus emerging from the literature that pairs of enantiomeric compounds may originate from two distinct biosynthetic pathways<sup>6</sup>. Thus, the number of resulting enantiomer mixtures relies on the number of stereocentres affected by racemases/epimerases. The pyrrolidinone-containing flavonoids such as dracocephins/drahecephins (**39**) (FIG. 4c) have been isolated as mixtures of pairs of enantiomers for flavones<sup>23</sup> and enantiomeric diastereomers for flavanones<sup>36,104</sup> from *Dracocephalum* sp. and *Scutellaria moniliorrhiza*.

Other phenolic compounds have similar biosynthetic origins<sup>70,124</sup>, for example longamides and related analogues originate from amino acids. Racemases and epimerases have been revealed for amino acids<sup>117,122</sup> and carbohydrates<sup>123</sup>. However, to fully understand the racemization of secondary metabolites, one should start considering that the traditional enzymes involved in the metabolisms of metabolites — synthases,

oxidoreductases, transferases, hydrolases, lyases — are also all potential isomerase enzymes. This assumption is consistent with popular thought, championed by Tanner<sup>10</sup>, that any enzyme able to induce bond cleavage at a stereogenic centre could potentially catalyse its epimerization. For instance, enzymatic interconversion of configurations is a key argument in the literature to explain racemization and enantiomer occurrence in the benzyloquinoline alkaloids series<sup>125</sup>. Conversely, the opposite species, enantioselective enzymes, are found in plants and other organisms<sup>6</sup>. Therefore, stereoisomers of opposite handedness could be biosynthesized by mirror-image enzymes present in certain organisms<sup>6</sup>. For example, germacrene D synthases of opposite handedness have been characterized from the plant *Solidago canadensis* and, interestingly, both enantiomers of this compound occur in the same species<sup>126,127</sup>.

Few natural enzymes that are dedicated to the transannular cyclohexene ring formation through Diels–Alder cycloaddition reactions have been reported. Most of these cycloaddition-inducing enzymes, or cyclases, intervene in other biological processes with a successive reduction of their catalytic efficacy, whereas few are stand-alone enzymes. Hashimoto and Kuzuyama carried out a comprehensive study to determine the nature of these enzymes<sup>11</sup>. The discovery of these cyclases changed the understanding of cycloadditions and revolutionized the discussions about the biosynthetic origins of racemates. However, the recognized success of these groups of enzymes was limited to intramolecular cycloaddition. Recently, Gao et al. isolated the first ever reported intermolecular enzyme from *Morus alba*, named *Morus alba* Diels-alderase (MaDA) (FIG. 4d), responsible for concerted pericyclization. This is exemplified by the proposed formation of hyperjapone A (**40**) in *Hypericum japonicum*, between diene and dienophile entities with exceptional catalytic and stereospecific efficiency<sup>128</sup>.

Racemization processes in secondary metabolism may include both homodimerization and heterodimerization. Most dimerization events follow oxidative coupling strategies<sup>56</sup>, with the exception of meroterpenes and polymeric stilbenes, where cycloaddition governs the process. Polyflavonoids are one such example, with a mechanism that involves radical coupling. The meroterpenes, such as hypulatone B (**41**) for instance, were tentatively assigned to be generated from a radical coupling rather than cycloaddition, as is common in this group<sup>56</sup> (FIG. 4e). However, although oxidative coupling may explain dimerization in this case, it cannot support the racemization of these molecules alone, as the mechanism only affects one stereocentre. Most lignans are generated by the oxidative coupling pathway involving oxidative enzymes (for example, peroxidases or laccases) under the influence of dirigent proteins (DIRs), which are proteins that mediate the regioselectivity and stereoselectivity, leading to stereospecific products. If DIRs of opposite handedness are present within the same organism, this would result in the formation of racemates<sup>129–131</sup>. Indeed, the lack of strict stereospecific/stereoselective enzymatic control can lead to mixtures, including the formation of pairs of enantiomers in equal (racemic) or different ratios (scalemic). In lignan biosynthesis, for example,

Table 2 | Some biomimetic syntheses of racemates to explain non-enzymatic racemization pathways

Main class of racemates	Examples	Proposed biosynthesis routes (creation of racemates when applicable)
Meroterpenes	Guajadial B <sup>135</sup>	Hetero-Diels–Alder cycloaddition for the hybrid condensation and Knoevenagel condensation for the phenyl fixation
Alkaloids	Meyeniins A–C <sup>164</sup>	Racemization at C-7a during condensation catalysed by pyridine, then epimerization at C-3 by the Edman degradation reaction
	Setigerumine I	Intramolecular 1,3-dipolar cycloaddition
	Dactylicapnosinine Dactylicapnosine <sup>165</sup>	
Polyketides	Kingianin A <sup>166</sup>	Stereospecific electrocyclicization to a bicyclo[4.2.0]octadiene which then undergoes Diels–Alder reaction
	Epicolactone <sup>167</sup>	Racemization arises from a heterodimerization related to [5 + 2] cycloaddition of two <i>o</i> -quinones
	Homodimericin A <sup>168</sup>	Non-enzyme-catalysed six-electron cascade sequence of oxidation, Michael reaction/aromatization, intramolecular quinone-based Diels–Alder reaction and intramolecular Prins cyclization of two hydroquinone monomers
	Santalol Y <sup>169</sup>	[2 + 3] cycloaddition of a benzyl styrene and a vinyloxidopyrylium ion from an isoflavylum
	Goupiolone B <sup>170</sup>	Oxidative condensation via a benzobicyclo[3.2.1]octane intermediate followed by an intermolecular 1,4-addition, then intramolecular 1,2-addition and reduction
	Ocellatusones <sup>171</sup>	Photo-catalysed isomerization, electrocyclicization, [1,3]-sigmatropic shift and Claisen condensation
Miscellaneous	Dracocephins A and B <sup>172</sup>	Flavonoid–pyrrolidone racemates arise from a Strecker degradation of the amino acid L-glutaminethrough an alkylating receptor <i>N</i> -acyliminium ion and following the flavonoid formation
	Incarviditone	Heterochiral and homochiral oxa-Michael dimerizations
	Incarvilleatone <sup>173</sup>	

in vitro experiments revealed that in the absence of DIRs, laccases or peroxidases catalyse the dimerization (phenoxy radical coupling) of two (*E*)-coniferyl alcohol derivatives leading to a mixture of (±)-8,8', (±)-8,5' and (±)-8-*O*-4-linked racemic products (that is, lack of regioselectivity and stereoselectivity)<sup>130,132</sup>. Interestingly, in the presence of a DIR, the 8,8' product is formed preferentially, yielding only the (+)-8,8'-linked (when mediated by (+)-DIR) or (–)-8,8'-linked (when mediated by (–)-DIR) product<sup>131</sup>. DIRs of opposite stereoselectivities, (+)-DIR and (–)-DIR, were first reported from plants<sup>132,133</sup>. Thus, 8,8'-linked and predominantly racemic lignans such as (±)-syringaresinol (**42**) in *Tamarix nilotica* could hypothetically originate from the intervention of two distinct DIRs, (+)-DIR/(–)-DIR (FIG. 4f). The evidence above establishes that nature may contain many more undiscovered materials, including enzymes and DIRs, and finding their roles in metabolic processes may open new avenues in science.

### Pharmacological significance

Chiral compounds continue to fuel the pipeline of drug discovery against a panel of diseases and constitute ~50% of marketed drugs, out of which >80% are marketed as racemates<sup>134</sup>. From a biological standpoint, naturally occurring racemates examined in the course of this survey have shown considerable anticancer (**7**, **43**, **44**)<sup>135,136</sup>, antimicrobial (**10**, **22**, **45**, **46**)<sup>77,137–139</sup>, antiviral

(**47**, **48**)<sup>84,140</sup>, anti-inflammatory (**21**, **49**)<sup>141,142</sup>, antioxidant (**24**, **25**, **50**)<sup>82,143</sup>, antiprotozoal (**51**, **52**)<sup>73,144,145</sup>, anti-diabetic (**54**, **55**)<sup>146,147</sup> and other medicinally relevant activities (FIG. 5).

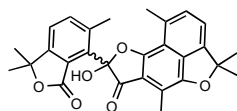
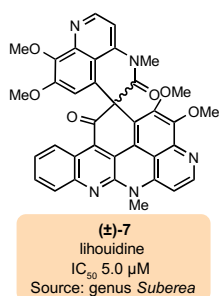
The majority of the racemates examined with biological activities were based on meroterpene, polyketide and alkaloid scaffolds. Meroterpenoids originate through coupling of terpene with either an acylphloroglucinol derivative<sup>148</sup> or 3,5-dimethylorsellinic acid<sup>149</sup>. Phloroglucinol derivatives are well known for their significant antibacterial effect<sup>150,151</sup>. However, sesquiterpenoid-based meroterpenoids bearing an acylphloroglucinol skeleton such as drycham-pone A (**14**) showed virtually no activity against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) and a bacterial plant pathogen (*Dickeya zeae*)<sup>54</sup>. Several polyketides are clinically important with a broad spectrum of activities mainly against infectious microbes<sup>152</sup>. Cratosumatranone D (**22**), a racemic polyketide, shows potent antibacterial activity against *Micrococcus luteus*, *Bacillus cereus*, *B. subtilis*, *S. aureus*, *Staphylococcus epidermidis*, *E. coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* with minimum inhibitory concentration (MIC) values of 8, 8, 16, 32, 8, 64, 32 and 16 µg ml<sup>-1</sup>, respectively<sup>153</sup>. Racemic alkaloids represent a class of secondary metabolites known for their intrinsic and extrinsic mechanism of action against cancer cell

lines<sup>154,155</sup>. Pyrisulfoxin D (**44**) displays excellent cytotoxicity against 26 out of 27 (96.3%) cancer cells lines tested, with IC<sub>50</sub> values ranging from 0.92 to 9.71 μM<sup>136</sup>.

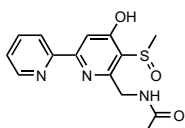
In general, one enantiomer may be more effective than the other at eliciting a biological response. This results in drug companies having a preference for single-enantiomer clinical compounds because they have potential advantages such as high therapeutic index and no chiral inversion (that is, chiral stability),

leading to reduced negative side effects and highly selective pharmacodynamic properties compared with the mixture of both<sup>115,156</sup>. However, some racemic mixtures display synergetic activities when interacting with drugs. For instance, racemates such as antineoplastic agent involucratasin E (**43**) show interaction effects on doxorubicin, increasing its cytotoxicity by 2.2 times against a breast cancer cell line and providing a new lead compound for clinical study<sup>86</sup>.

### a Antineoplastic effects

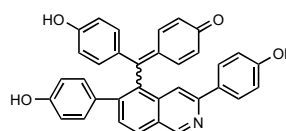


(±)-43  
involucratasin E  
increases doxorubicin IC<sub>50</sub>  
Source: *Stahlianthus involucratus*

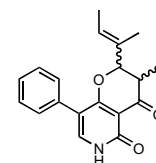


(±)-44  
pyrisulfoxin D  
IC<sub>50</sub> 0.92–9.71 μM  
Source: *Streptomyces albolongus*

### b Antifungal effects

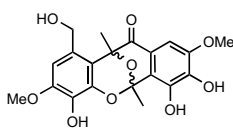


(±)-45  
selaginisoquinoline A  
MIC<sub>80</sub> 16–32 μg ml<sup>-1</sup>  
Source: *Selaginella pulvinata*

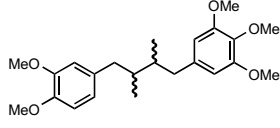


(±)-46  
citridone A  
MIC 32–128 μg ml<sup>-1</sup>  
Source: *Penicillium* sp.

### c Antiviral effects

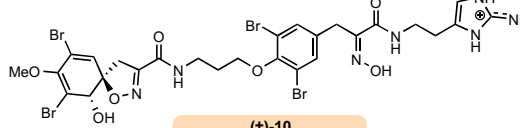


(±)-47  
integrastatin A  
IC<sub>50</sub> 0.6–1.1 μM  
Source: unnamed fungus  
ATCC74478

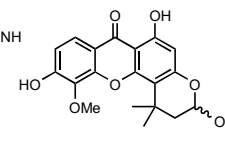


(±)-48  
tiegusanin N  
EC<sub>50</sub> 9.4 μM  
Source: *Schisandrapropinqua* var.  
*sinensis*

### d Antibacterial effects

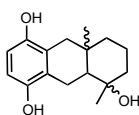


(±)-10  
purealin  
MIC 0.8–3.8 μM  
(–)-enantiomer given  
Source: *Pseudoceratina*  
marine sponge

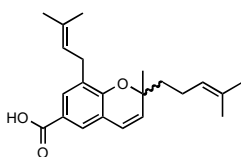


(±)-22  
cratosumatranone D  
MIC 8–64 μg ml<sup>-1</sup>  
Source: *Cratoxylum*  
*sumatranum*

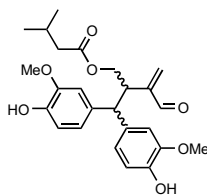
### e Antiprotozoal effects



(±)-51  
cordiaquinol J  
IC<sub>80</sub> 1.8–3.0 μg ml<sup>-1</sup>  
Source: *Cordia fragrantissima*

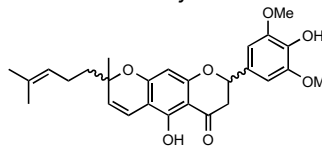


(±)-52  
gaudichaudianic acid  
IC<sub>50</sub> 4.2–6.1 μM  
Source: *Piper gaudichaudianum*

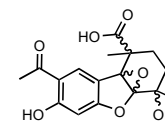


(±)-53  
brachangobinan B  
IC<sub>50</sub> 2.8 μM  
Source:  
*Brachanthemum gobicum*

### f Anti-inflammatory effects

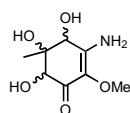


(±)-21  
paulownione C  
IC<sub>50</sub> 0.4 μM  
Source: *Paulownia tomentosa*

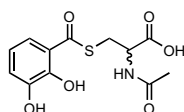


(±)-49  
eupatonin A  
IC<sub>50</sub> 8.6 μM  
Source: *Eupatorium chinense*

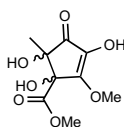
### g Antioxidant effects



(±)-24  
EC<sub>50</sub> 8.2 μM  
Source: *Alternaria* sp.

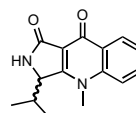


(±)-50  
ED<sub>50</sub> 13.0 μM  
Source: marine  
*Actinomyces* strain

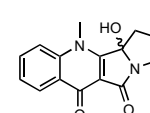


(±)-25  
EC<sub>50</sub> 16.1 μM  
Source: *Alternaria* sp.

### h Antidiabetic effects



(±)-54  
quinolactacin B  
IC<sub>50</sub> 57.3 μM  
Source: *Penicillium* sp.



(±)-55  
oxypenicinoline A  
IC<sub>50</sub> 317.8 μM  
Source: *Penicillium steckii*

Fig. 5 | **Chemical structures of racemic natural products with biological activities.** Labels of chemical structures are coloured to highlight the sources in nature: green for plants, purple for microorganisms and orange for animals. **a–h** | Compounds within the same shape are the most active racemates (evaluated by the values of IC<sub>50</sub>, EC<sub>50</sub>, minimum inhibitory concentration (MIC) or MIC<sub>80</sub>) of the entitled pharmacological activities as reported in the literature for antineoplastic (lihouidine (**7**)<sup>31</sup>, involucratasin E (**43**)<sup>86</sup>, pyrisulfoxin D (**44**)<sup>136</sup>) (part a), antifungal (selaginisoquinoline A (**45**)<sup>77</sup>, citridone A (**46**)<sup>84</sup>) (part b), antiviral (integrastatin A (**47**)<sup>140</sup>, tiegusanin

N (**48**)<sup>182</sup>) (part c), antibacterial (purealin (**10**)<sup>34</sup>, cratosumatranone D (**22**)<sup>153</sup>) (part d), antiprotozoal (cordiaquinol J (**51**)<sup>144</sup>, gaudichaudianic acid (**52**)<sup>159</sup>, brachangobinan B (**53**)<sup>73</sup>) (part e), anti-inflammatory (paulownione C (**21**)<sup>177</sup>, eupatonin A (**49**)<sup>183</sup>) (part f), antioxidant ((4*R*\*,5*S*\*,6*S*\*)-3-amino-4,5,6-trihydroxy-2-methoxy-5-methyl-2-cyclohexen-1-one (**24**)<sup>82</sup>, (4*S*\*,5*S*\*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten-1-one (**25**)<sup>82</sup>, 2-acetamido-3-(2,3-dihydroxybenzoylthio)propanoic acid (**50**)<sup>184</sup>) (part g) and antidiabetic (quinolactacin B (**54**)<sup>146</sup>, oxypenicinoline A (**55**)<sup>147</sup>) (part h) activities.

Considering the concept of 'one drug one target' and considering that the human body is incredibly chiral selective, administration of racemates implies treatment of diseases with two drugs with different pharmacokinetic, pharmacological and pharmacodynamic profiles. Moreover, in the pharmacology field, racemic drugs can be classified into three groups. The first group represents racemates containing one active ingredient, or eutomer, and its enantiomer counterpart, or distomer, which produces a less active/toxic or different therapeutic effect. This can be exemplified by the synthetic racemic drug citalopram, with only its (S)-enantiomer carrying the desired therapeutic benefits<sup>14</sup>. The second group consists of racemic drugs constituted by two enantiomers of equal therapeutic profiles, such as for the drug fluoxetine<sup>157</sup>. The last group includes drugs with chiral inversion properties, with one or both enantiomer(s) prone to undergo racemization or enantiomerization, such as ketoprofen and thalidomide<sup>13</sup>.

Besides their effective treatment of numerous diseases, many existing racemic drugs have been associated with chronic side effects. As a result, many racemic drugs have witnessed a strategy known as the chiral switch (that is, replacement of a drug approved as a racemate with a drug containing only one of the two enantiomers in the previous version), increasing the arsenal of enantiomeric drugs. For example, chloroquine and its analogue hydroxychloroquine are well-known antimalarial drugs administered as racemates but result in negative side effects. Switching to a single enantiomer, the dextrorotatory (eutomer) isomer was found to be more effective for the management of COVID-19 (REF.<sup>158</sup>).

In summary, chirality in drug discovery programmes represents a blueprint for the future generation of drugs. The pharmacological effect of each enantiomer in the racemic mixture often differs in a chiral environment. Thus, the choice to make up a drug of either the racemate or a single enantiomer must take into consideration many clinical assessments, as mixture of enantiomers can induce synergistic, additive, reduced or negative effects. For example, the natural racemate cnidimonin A (**28**) ( $IC_{50} = 1.23 \mu\text{M}$ ) has a greater antiviral potency than its single enantiomers ( $IC_{50} \geq 3.7 \mu\text{M}$ )<sup>81</sup>, and gaudichaudianic acid (**52**) ( $IC_{50} = 55.8 \mu\text{M}$ ) has a much increased trypanocidal activity than its single enantiomers ( $IC_{50} > 176.0 \mu\text{M}$ )<sup>159</sup>.

## Conclusion

Overall, racemates occur in every organism on Earth and constitute one of the most intriguing topics in science. Their origin, however, is still deeply discussed. Racemic natural products are most commonly generated from 'non-enzymatic' routes. As enzymes typically form single enantiomers, the occurrence of racemates from enzymatic pathways in nature is still difficult to comprehend. The characterization of enzymes which can generate racemic mixtures and the investigation of pathway promiscuity towards racemic intermediates are important areas towards a better understanding of natural racemate occurrence. The discovery of many more racemates or the analysis of intact cells appears to be a good strategy to explore this issue.

Recognizing the presence of racemates upon isolation of natural products is an area for improvement. For instance, there is no optical rotation threshold in the literature, from which an isolated compound can be claimed to be racemic. Moreover, circular dichroism spectroscopy, crucial for the absolute configuration determination, still relies on complex quantum mechanics calculations. This method often requires comparison of both experimental and calculated circular dichroism spectra which is not readily available in some laboratories. The structure elucidation of more racemates including the absolute configuration of both enantiomers must be achieved with a simple technique without DFT methods that are not always affordable. Recently, the crystalline sponge method was developed for X-ray analysis and has already demonstrated several applications in natural product discovery<sup>112</sup>. Additionally, the MicroED technique has recently been developed as a new promising alternative to XRD for structure determination<sup>108–111</sup>. The combination of these existent tools and the development of new ones, especially the advanced techniques, for a fast resolution and structure elucidation of small amounts of racemates will significantly advance natural product research and drug discovery. Finally, natural products remain an important source of therapeutically relevant chiral compounds supplying drug discovery pipelines with both enantiomerically pure compounds and racemates. Although natural racemates have historically been underexplored, they have great promise in the future of drug discovery.

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#### Author contributions

All authors collected data, wrote and edited the manuscript. G.T.M.B. and V.-A.N.-N. drafted the manuscript. D.M. provided substantial content. S.A.F. conceived the content, and edited and finalized the manuscript.

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