Linear Naphtho-γ-Pyrones: A Naturally Occurring Scaffold of Biological Importance

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Abstract: The linear naphtho-γ-pyrone (LNGP) moiety is a naturally occurring scaffold with broad biological activity ranging through antimicrobial, antiviral, insecticidal and anti-estrogenic activity. This review, the first of its kind, surveys the chemical literature in an effort directed toward assembling data that will facilitate the construction of activity profiles associated with this emerging class of compounds. The structural and associated biological information has been presented in tabular format with all structures revealed throughout the document and referencing that will allow the reader to rapidly access the literature pertaining to a specific activity or structural class.

INTRODUCTION

Naphthopyrones combining a naphthalene and an α, β or γ-pyrone, where the Greek letter refers to the position of the oxygen atom in the pyrone *vis-à-vis* the carbonyl group, exist in 18 isomeric forms collectively referred to as naphthopyrones (NPs) (Fig. **1**). These structural units may be organised so as to create either an angular arrangement of the naphthalene and pyrone moieties (Fig. **1a**) or the alternative linear arrangement (Fig. **1b**). Whereas angular naphthopyrones (ANPs) may exist in two angular isomeric forms as benzo[*f*]-chromenones or -isochromenones and benzo[*h*]-chromenones or -isochromenones accounting for 12 of the isomers, linear naphthopyrones (LNPs), benzo[*g*]-chromenones or -isochromenones, are further constrained and account for the remaining 6 isomers (Fig. **1**). Of the 18 possible isomeric forms three can exist as naphtho-γ-pyrones (NGPs), of which two are angular naphtho-γ-pyrones (ANGPs), while one possesses the linear naphtho-γ-pyrone (LNGP) skeleton forming the basis of this review. Fig. (**1**) shows the various NP skeletons and their names employing the widely used chromenone/isochromenone system of nomenclature (shown in italics) in addition to their CA Index name (shown in plain font) beneath each structure. Where dashed bonds occur within a structure it indicates the possibility for the compound to exist as either an unsaturated or a dihydro form. In such cases only the name for the unsaturated member is provided for each structural class.

Surveying the literature recognises that many more examples of ANGPs are known than LNGPs and this can be linked to the fact that the majority of ANGPs are synthetically produced, and regiochemically these compounds are easier to prepare than their linear counterparts. However, the LNGPs represent a frequently encountered, naturally occurring scaffold with a diverse range of biological activities. While conducting research into biologically active LNGP based natural products we recognised a significant gap in the literature relating to this class of compound. The LNGP scaffold has been structurally known for over 50 years with the first member of this class, rubrofusarin (**1**), being isolated in 1937 and structurally identified in 1961 [1, 2]. History further reveals that biological activity possessed by LNGPs was known well before the chemistry had evolved to a sophistication capable of allowing structural elucidation, with the red pigment from *Ustilaginoidea virens* (a rice fungus) being recognised as possessing antimicrobial activity as far back as 1895 [3]. The compounds ultimately responsible for the observed activity were later shown to be a series of dimeric LNGPs now recognised as ustilaginoidins (e.g. ustilaginoidin A **41**) [4]. The rate at which novel LNGPs have been isolated or synthetically produced is steadily growing as is the literature associated with LNGP biological activity. We anticipate this review will draw attention to the LNGP class as a naturally occurring scaffold of biological importance.

BIOSYNTHETIC ORIGINS

Investigations into the biosynthesis of LNGPs have shown they are produced *via* the polyketide pathway. These studies have centred on four LNGPs, namely rubrofusarin (**1**), fonsecin (**6**), chaetochromin A (**49**), and YWA1 (**145**), utilising traditional acetate feeding experiments to unravel mechanistic detail. More recently YWA1 has also been the subject of a number of gene-based investigations [5-17]. From a knowledge of polyketide biosynthesis it follows that the prevalence for the formation of α- and γ-pyrones in either angular or linear form will predominate over β-pyrones, and this is borne out by examination of the chemical literature. Given that several species of fungi that produce LNGPs also demonstrate the capacity to produce ANGPs, the question remains open as to whether distinct biosynthetic machinery is in place to selectively produce angular *versus* linear NPs.

Examination of the heptaketide backbone indicates two possible folding patterns exist that would achieve the desired LNGP skeleton present in rubrofusarin. Staunton and col-

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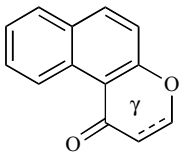
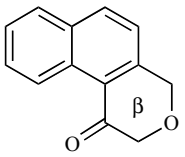
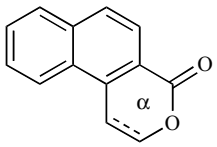


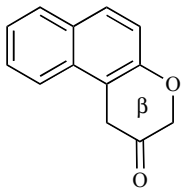
1H-benzo[f]chromen-1-one
1H-naphtho[2,1-b]pyran-1-one



2H-benzo[f]isochromen-1(4H)-one
2H-naphtho[2,1-c]pyran-1(4H)-one



4H-benzo[f]isochromen-4-one
4H-naphtho[2,1-c]pyran-4-one



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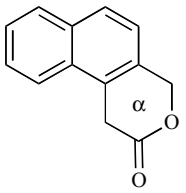
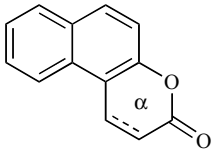
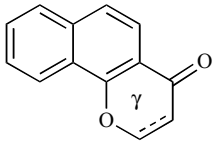
1H-naphtho[2,1-b]pyran-2(3H)-one



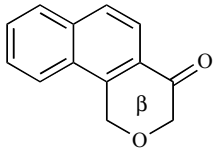
1H-benzo[f]isochromen-2(4H)-one
2H-naphtho[2,1-c]pyrano-2-one, 1,4-dihydro-



3H-benzo[f]chromen-3-one
3H-naphtho[2,1-b]pyran-3-one



4H-benzo[h]chromen-4-one
4H-naphtho[1,2-b]pyran-4-one



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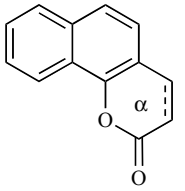
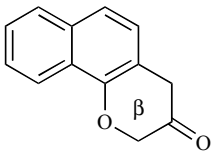
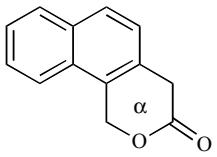
1H-naphtho[1,2-c]pyran-4(3H)-one



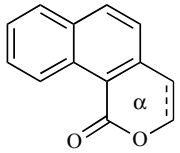
2H-benzo[h]chromen-2-one
2H-naphtho[1,2-b]pyran-2-one



2H-benzo[h]chromen-3(4H)-one
2H-naphtho[1,2-b]pyran-3(4H)-one

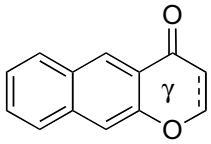


1H-benzo[h]isochromen-3(4H)-one
3H-naphtho[1,2-c]pyran-3-one, 1,4-dihydro-

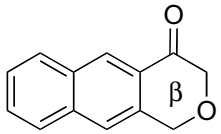


1H-benzo[h]isochromen-1-one
4H-naphtho[2,1-c]pyran-4-one

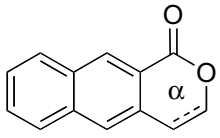
Fig. (1a). The 12 angular isomeric forms of naphthopyrones (ANPs).



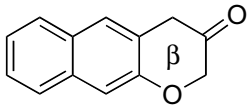
4H-benzo[g]chromen-4-one
4H-naphtho[2,3-b]pyran-4-one



1*H*-benzo[*g*]isochromen-4(3*H*)-one
1H-naphtho[2,3-c]pyran-4(3H)-one



1H-benzo[g]isochromen-1-one
1H-naphtho[2,3-c]pyran-1-one



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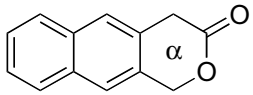
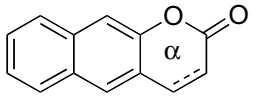
4H-naphtho[2,3-b]pyran-3-one



1H-benzo[g]isochromen-3(4H)-one
3H-naphtho[2,3-c]pyran-3-one, 1,4-dihydro-



2H-benzo[g]chromen-2-one
4H-naphtho[2,3-b]pyran-2-one

Fig. (1b). The 6 linear isomeric forms of naphthopyrones (LNPs).

Fig. (1). The 18 isomeric forms of naphthopyrones (NPs).

leagues utilised the fungus, *Fusarium culmorum* in a number of feeding experiments employing labelled acetate to definitively demonstrate that *F. culmorum* produced rubrofusarin (**1**) *via* Path B (Fig. **2**) [5, 8, 18].

In addition to the monomeric LNGPs a large number of naturally occurring dimeric linear naphtho-γ-pyrones (DLNGPs) are known. The most probable biogenesis of these metabolites is through phenolic oxidative coupling of polyketide-derived monomeric subunits and as such several different structural forms are possible. The majority of these molecules contain two LNGP subunits and are therefore *true dimers*. The minority of compounds contain a single LNGP subunit, but have a second structurally related group, such as an ANGP or a naphthoquinone and are considered here as *pseudodimers* of DLNGPs. The true dimers can exist as homo- or hetero- dimers, with symmetric or unsymmetric bridging. Symmetric bridged dimers share bridging atoms across equivalent positions on each LNGP moiety whereas

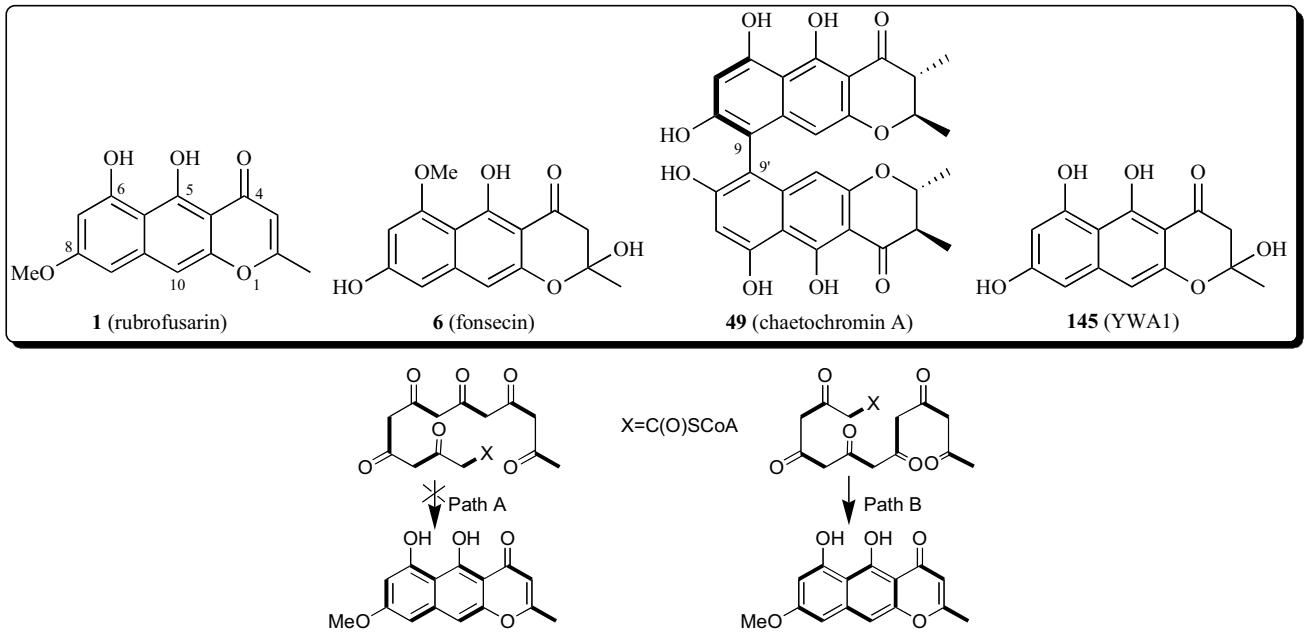


Fig. (2). Possible folding patterns of the heptaketide leading to rubrofusarin (**1**).

unsymmetric bridged dimers share different bridging positions. Provided one true LNGP subunit exists in a dimer we have employed the term DLNGP (Fig. **3**).

BIOLOGICAL ACTIVITY ASSOCIATED WITH NATURALLY OCCURRING LNGP

In addition to the antibiotic properties displayed by the rice fungus *Ustilaginoidea virens* another early glimpse into the biological properties exhibited by LNGP can be found in work reported by Sciarini and co-workers in 1943. They found that when rubrofusarin (**1**) was added to a growing culture of the fungus *Fusarium lini* imbued with isopropanol, the rate of transformation of isopropanol to acetone was diminished; conversely addition of some simple xanthones or nicotinic acid increased this rate [19]. Few reports of biological activities in this class occur after this until the 1970s;

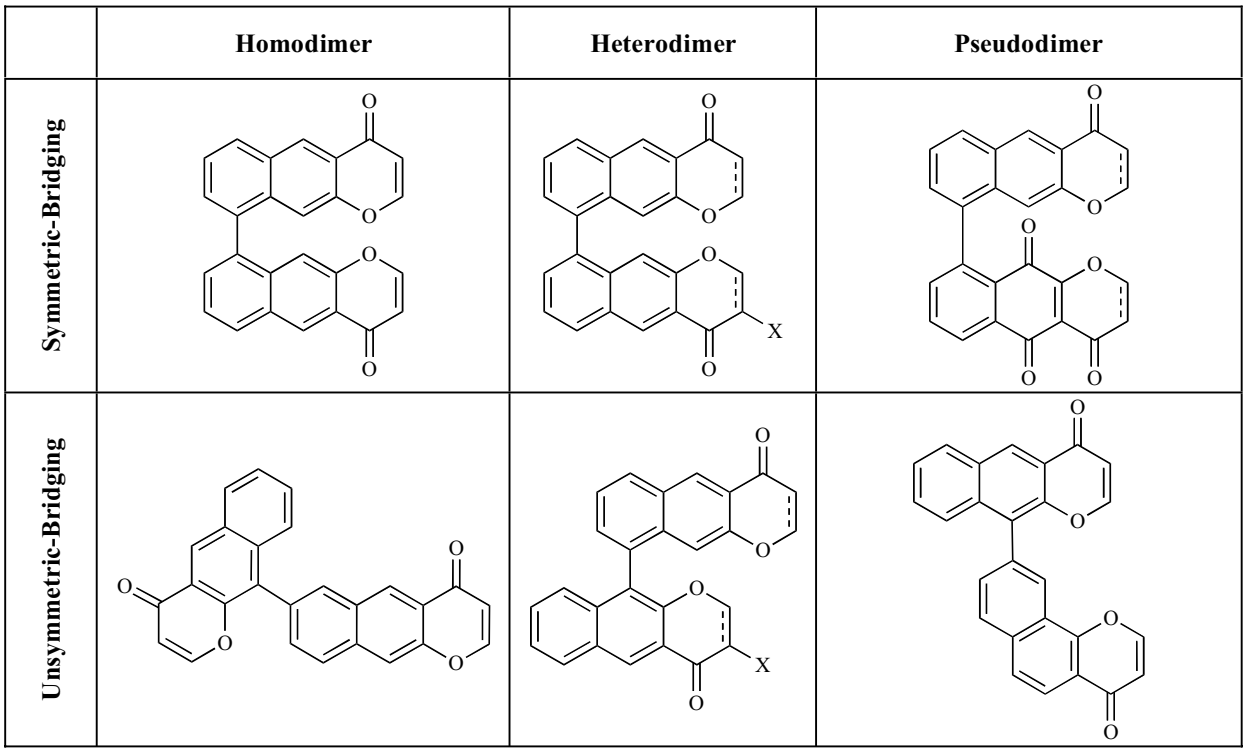


Fig. (3). Dimeric linear naphtho-γ-pyrones (DLNGP) structural variations.

Table 1. Structures and Associated Biological Evaluations of LNGPs

		1	2	3	4	5	6	7	8	9
Compound Number	**Compound Name**	**Antimicrobial**	**Antifungal**	**Antiviral**	**Phytotoxic**	**Anticancer**	**Cytotoxicity (General)**	**Insecticidal**	**Miscellaneous Biological Assay *(see Table 2)***	**References**
Monomeric linear naphtho-γ-pyrones (LNGPs)										
1	rubrofusarin	A, N	N			A	A		1N, 19A,*	[19-28]
2						N				[27]
3	*nor*-rubrofusarin	N	N			A			19A	[20, 25, 29]
4						N				[27]
5	rubrofusarin B	A	A		A	A	N		2N, 15A	[29-36]
6	fonsecin								2A	[28, 35]
7	fonsecin B								2N	[35]
8	carbonarin A							A		[37]
9	carbonarin C							A		[37]
10	carbonarin F							A		[37]
11	carbonarin G							A		[37]
12	carbonarin H							A		[37]
13-19					A					[32-34]
20	cassiaside	N	N						3A, 16A	[25, 38-41]
21		N							3A, 16A, *	[23, 38, 39, 41, 42]
22	cassiaside B	A, N	N							[25, 43]
23									16A	[41]
24	cassiaside B_2								4N	[44]
25		N								[23]
26	nigerasperone A	N				N				[45]
27	quinquangulin	A, N	N			A				[22, 25, 27]
28-29						N				[27]
30		A, N	N							[25, 43]
31		A, N	A, N							[25, 43]
32	adenaflorin A					A				[46]
33	adenaflorin B					A				[46]

(Table 1. Contd….)

		1	2	3	4	5	6	7	8	9
Compound Number	**Compound Name**	**Antimicrobial**	**Antifungal**	**Antiviral**	**Phytotoxic**	**Anticancer**	**Cytotoxicity (General)**	**Insecticidal**	**Miscellaneous Biological Assay *(see Table 2)***	**References**
34	comantherin								17A	[47]
35	neocomantherin								17A	[47]
36	neocomantherin sulfate								17A	[47]
37	YMC-256A1								20A	[48]
38-39									20A	[48]
Dimeric linear naphtho-γ-pyrones (DLNGPs)										
40	ustilaginoidin C					N				[29]
41	ustilaginoidin A					A	A			[29, 49, 50]
42	ustilaginoidin B					A				[29]
43	cephalochromin	A, N	N			A			*	[29, 50-56]
44-45									*	[53]
46	dihydroisoustilaginoidin A	A, N	N							[54]
47	dihydroustilaginoidin A	A, N	N			A			*	[29, 53-54]
48									*	[57]
49	chaetochromin A					A	A		6N, *	[29, 49, 50, 53, 58-67]
50						A			*	[29, 53]
51						N				[29]
52						A				[29]
53						A				[29]
54						N				[29]
55	chaetochromin B					A				[29]
56	chaetochromin C					A				[29]
57	chaetochromin D					A	A			[29, 49, 63]
58	ustilaginoidin D	A				A				[29, 68]
59	ustilaginoidin E					A				[29]
60	ustilaginoidin G					A				[29]
61	ustilaginoidin H					A				[29]
62	ustilaginoidin I					A				[29]

(Table 1. Contd….)

		1	2	3	4	5	6	7	8	9
Compound Number	**Compound Name**	**Antimicrobial**	**Antifungal**	**Antiviral**	**Phytotoxic**	**Anticancer**	**Cytotoxicity (General)**	**Insecticidal**	**Miscellaneous Biological Assay *(see Table 2)***	**References**
63	ustilaginoidin J					A				[29]
64	isochaetochromin B_1			A						[69, 70]
65	isochaetochromin B_2			A						[69, 70]
66	isochaetochromin D_1			A						[69, 70]
67	oxychaetochromin B			A						[69, 70]
68-73				A						[69, 70]
74	ustilaginoidin K	A								[68]
75	ustilaginoidin L	A								[68]
76	aurasperone A	A	A			A			5N, 15A	[36, 71]
77	aurasperone B					N				[29]
78						A				[31]
79	aurasperone D						A			[21]
80	fonsecinone A	A	A			A			5A, 15A	[36, 71]
81									5A	[71]
82	fonsecinone A								5A	[71]
83		N	N							[72]
84	nigerone	A				A	N		*	[29, 30, 73-76]
85	isonigerone						N			[30]
86						N	N			[29, 30]
87	6'-*O*-demethylnigerone	A								[28]
88	8'-*O*-demethylisonigerone	A								[28]
89	asperpyrone D					N				[77]
90	nigerasperone C	A				N			3A	[45]
91	10,10'-bifonsecin B	N							*	[28, 55]
92-94									*	[55]
95									12A	[78, 79]
96-97									11A, 14A, *	[80-82]
98									11A	[81]

(Table 1. Contd….)

		1	2	3	4	5	6	7	8	9
Compound Number	**Compound Name**	**Antimicrobial**	**Antifungal**	**Antiviral**	**Phytotoxic**	**Anticancer**	**Cytotoxicity (General)**	**Insecticidal**	**Miscellaneous Biological Assay *(see Table 2)***	**References**
99									11N	[81]
100									11A	[81]
101									11A, *	[80-82]
102									11A	[81]
103									11N, *	[81-83]
104									7	[84]
105									18A	[85]
106									18A	[85]
107									18A, *	[85, 86]
108									8, 9N	[87, 88]
109-111									8	[87]
112									9N	[88]
113-118									18A	[89, 90]
119									9A	[88]
120						A	A		9A	[88]
121				A		A	A		9A	[88]
122-123									10N	[91]
124									20A	[48]
125-134									13	[92]
135-144									13	[92]

Note: 'A' implies the compound possesses the activity. This does not necessarily mean an assay is reported. For example, some patents describe a compound for use as an antiallergen, without provision of data.

KEY: A = active, N = inactive/non toxic

however, since then LNGPs have been subjected to a range of biological evaluations and found to display a diversity of activities (Tables **1**, **2** and **3**). Many of the biological studies have been performed on naturally occurring LNGPs or synthetic analogs and this is borne out through examination of the compounds contained in Table **1** where approximately 60% are of natural origin.

Of approximately 330 LNGPs known at least 144 of them have been subjected to biological evaluations and these are documented in Table **1**. The sequence of compound numbering throughout the text emanates from this table where compounds appear according to their broad structural classifications as either "monomeric linear naphtho-γ-pyrones (LNGPs)" (structures **1**-**39**) or "dimeric linear naphtho-γ-pyrones (DLNGPs)" (structures **40**-**144**). The table is relatively broad across each column, for example, general cytotoxicity (column 6) includes both cytotoxicity and teratogenicity; while anticancer activity (column 5) encompasses

activity against a variety of different cancer cell lines, and compounds with the capacity to reverse drug resistance in cancer cells. The most frequently encountered biological activities are shown in columns 1-7, where the results of the assays are summarised. There are many disparate biological assays/reports involving LNGPs that are also of interest and serve to highlight the remarkable activity possessed by this class. To demonstrate the wide array of biological evaluations and the medicinal significance of the LNGP structural class, miscellaneous assay types are shown in Table **2** and cross referenced to Table **1**. These assays are numbered and specific compounds that have been subjected to these assays are shown with the corresponding number in column 8 of Table **1**. Where a compound is active in the assay, the assay number is followed by an A (e.g. 1A), where it has been tested and found to be inactive an N is used (e.g. 1N), where there is ambiguity only the number is given. The remaining miscellaneous biological evaluations are shown with an asterisk and the reader is directed to the reference for a comprehensive assessment of the testing conducted. We have attempted to exhaustively collate biological activity belonging to the LNGP structural class, however, space considerations have necessitated some sacrifice regarding commentary. Where the following text lacks commentary concerning a particular compound the reader is directed to the appropriate reference.

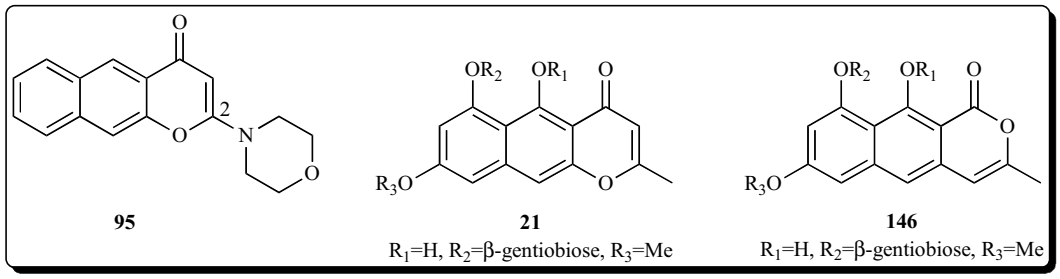
CONSIDERATIONS OF LNGP DRUG VIABILITY

While the LNGP structural motif has shown potent and promising biological activities in the laboratory, as far as we are aware its progression to the clinic has been limited. A small number of drug patents include some LNGP based compounds, yet it is not clear that any of these chemicals have obtained regulatory approval. Some LNGPs such as rubrofusarin (**1**) and associated glycosides (**20-22**), are found in the seeds of a number of *Cassia* sp. and these seeds are used as herbal remedies. For example, a patent claims natural rubrofusarin glycosides from *Cassia sp*. elevate glutathione levels *in vivo* [93].

It has been suggested that LNGPs may possess unwanted DNA intercalating properties, which could limit their desirability as therapeutic agents. Griffin and colleagues prepared the 2-morpholino substituted LNGP **95**, as part of a study looking for DNA dependent protein kinase inhibitors. Initial screens of various 2-amino-chromone structural types, in-

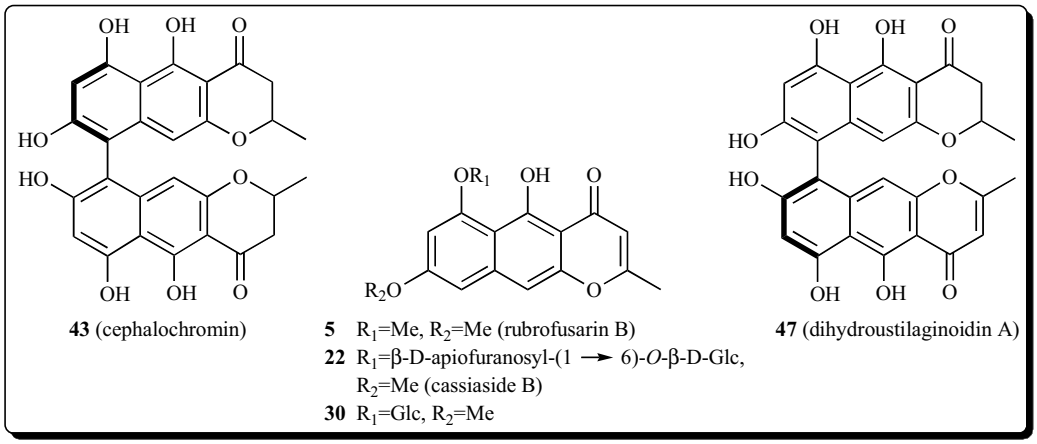
Table 2. Other Biological Assays Involving LNGPs

Assay Number	Miscellaneous Assay Type
1	cAMP phosphodiesterase inhibition [26]
2	Interleukin-4 transduction inhibition [35]
3	Radical scavenging activity [38, 40]
4	Inhibition of histamine release from mast cells [44]
5	Taq DNA polymerase inhibition [71]
6	Mutagenicity [67]
7	Antisecretory activity [84]
8	Anticonvulsive activity [87]
9	Inhibition of DNA and RNA synthesis [88]
10	Inhibition of induced human platelet aggregation [91]
11	Cystic fibrosis transmembrane conductance regulator activation [81]
12	DNA-dependent protein kinase inhibition [78, 79]
13	Modulation of protein tyrosine phosphatase [92]
14	Aromatase modulation [80]
15	Xanthine oxidase inhibition [36]
16	Antimutagenic [23, 24]
17	Fish Anitfeedant [47]
18	Antiallergenic [85, 90]
19	Estrogenic/anti-estrogenic activities [20]
20	Hypoxia inducible growth factor-1 (HIF-1) activity [48]
*	Other Miscellaneous Assays



cluding **95** and an angular variant, were found to possess strong activity (IC_{50} 0.39 and 0.23 μmol for the linear and angular variants respectively). However, due to the concerns about possible intercalation, no further LNGPs were prepared [78]. While the modes of action and structural requirements for biological activity in LNGPs have generally not been determined, the presence of the unsaturated pyrone moiety has been implicated in some cases. For example, the LNGP glycoside **21** is more active at protecting hepatocytes against galactosamine, than the equivalent linear naphtho-α-pyrone analogue **146** and this may be related to the capacity of this functional group to act as a Michael acceptor [94].

the biaryl axis, were found to be potent HIV integrase inhibitors with activity in the low μM range. The semi-synthetic acetate **68** and the methyl ether derivatives **69**-**73** all showed significantly lower activity which suggested free phenols are required for optimal activity in these compounds [69]. These inhibitors were initially reported in a patent which included dosage ranges but gave no cytotoxic evaluation or evidence of administration to animals [70]. A more recent patent describes a large number of phosphonate analogues of HIV integrase inhibitors and suggests DLNGPs could be derivatised as phosphonates [95]. To our knowledge this avenue of research has not been further developed.



1-2. ANTIMICROBIAL PROPERTIES

LNGPs have displayed activity against fungi, Gram-positive bacteria (including mycobacteria) and Gram-negative bacteria. The bacteria and fungi against which the LNGPs are active are summarised in Table **3**. It is interesting to note that all of the LNGPs shown in Table **3** are natural products or semi-synthetic derivatives thereof; these include both monomeric and dimeric LNGPs, for example rubrofusarin (**1**) and cephalochromin (**43**). Curiously no evaluations of antimicrobial activity for synthetic LNGPs have been reported, presumably due to the general neglect this class of compound has received from a synthetic perspective, despite reliable and well documented methods existing for their preparation.

3. ANTIVIRAL ACTIVITY: ANTI-HIV ACTIVITY - INTEGRASE INHIBITION

The DLNGP natural products **64**-**67**, which were all isolated from the same fermentation broth and contain examples of diastereomers possessing different configurations about

There are toxicity concerns associated with DLNGPs which would need to be overcome in order to develop a drug suitable for clinical use. These concerns are mentioned in section 6 (Cytotoxicity) as they pertain to other members of this class.

4. PHYTOTOXICITY

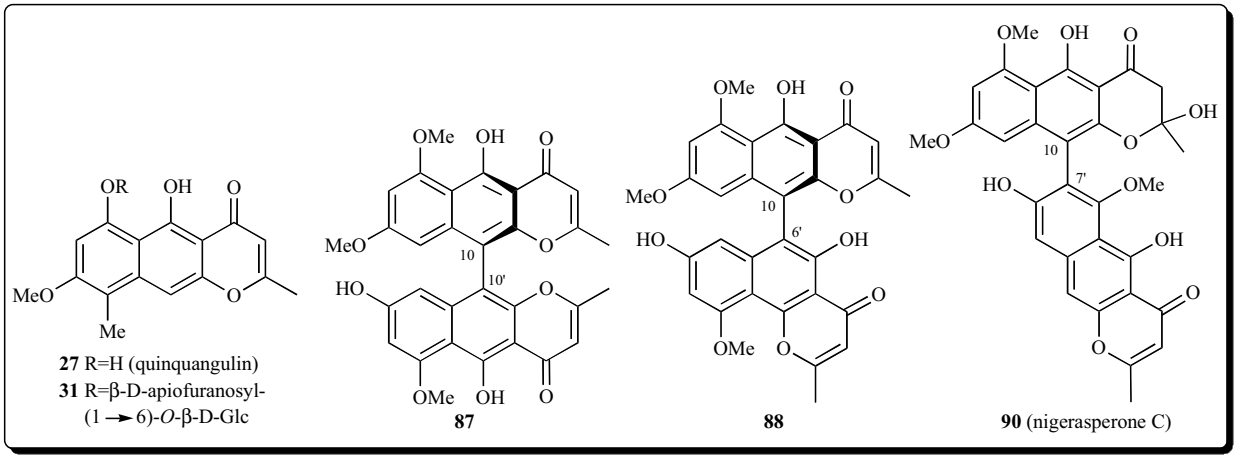
Phytotoxicity has not been correlated with the potential medicinal value of a compound. However, we are aware that therapeutically used compounds may manifest as environmental pollutants if they are phytotoxic and therefore consider this an important bioactivity to be noted for this class [96, 97]. The eight LNGPs obtained by Macías and colleagues from *Guanomyces polythrix* (**5**, **13**, **14**, **15**, **16**, **17**, **18**, and **19**) all demonstrated strong phytotoxicity against the plant species *Amaranthus hypochondriacus* and *Echinochloa crusgalli*. It was proposed the activity was derived by interference with a calcium binding protein, given the compounds were all demonstrated to modulate a calcium receptor [32-34].

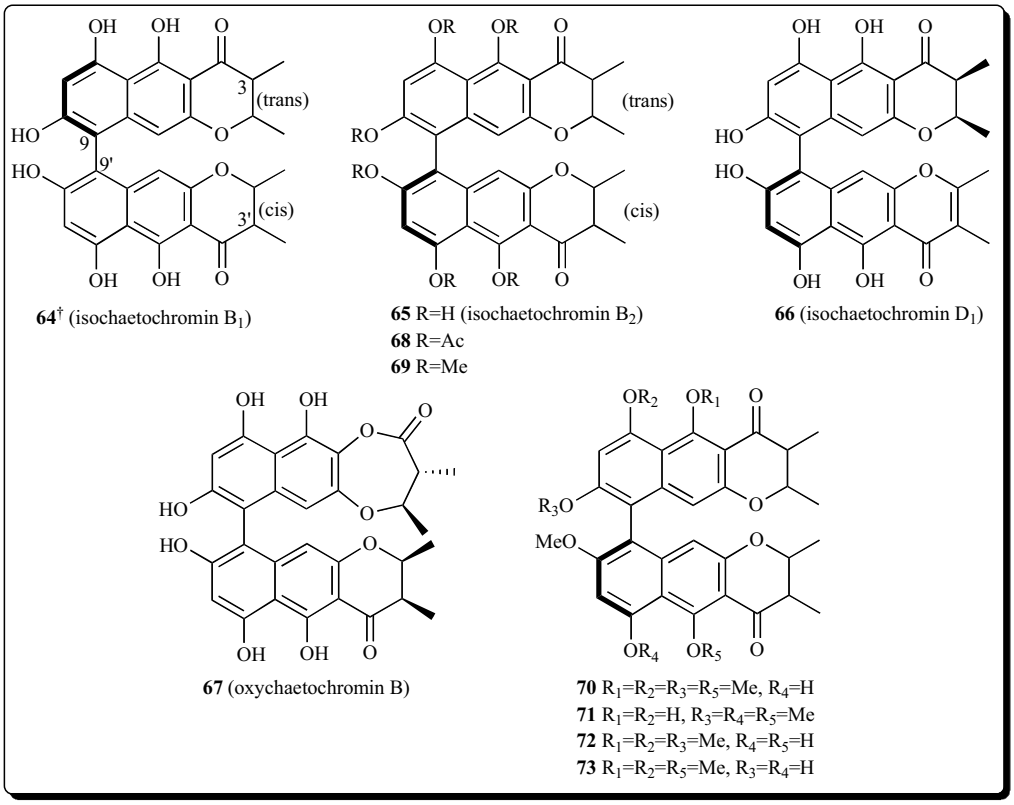
Table 3. LNGPs with Reported Antimicrobial Activities Against

	Compound number	*Staphylococcus aureus*	*Bacillus sp.*	*Streptococcus agalactiae*	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas fluorescens*	*Mycobacterium sp.*	*Acinetobacter sp.*	*Trichophyton rubrum (fungus)*	*Candida sp. (fungi)*	References
Monomeric LNGPs	**1**	A						A				[22-25, 28]
	5		A			A	A			A	A	[36]
	22		A			A	A		A	A	A	[43]
	27	A										[25]
	30								A			[43]
	31								A		A	[43]
Dimeric LNGPs	**43**	A	A		A							[51, 54]
	46	A	A		A							[54]
	47	A	A		A							[54]
	58	A										[68]
	74	A										[68]
	75	A										[68]
	76		A			A	A			A	A	[36]
	80		A			A	A			A	A	[36]
	84		A									[74-76]
	87							A				[28]
	88							A				[28]
	92										A	[45]

5. ANTICANCER ACTIVITY & STRUCTURE ACTIVITY RELATIONSHIPS

LNGPs have shown activity against a variety of cancer cell lines. Ogura and colleagues reported the first example of this, showing rubrofusarin (**1**) and quinquangulin (**27**) possessed moderate activity against P-388 cells but were inactive against 9KB cells. No activity was observed for the diacetate and dimethyl ether derivatives of either of these LNGPs [27].

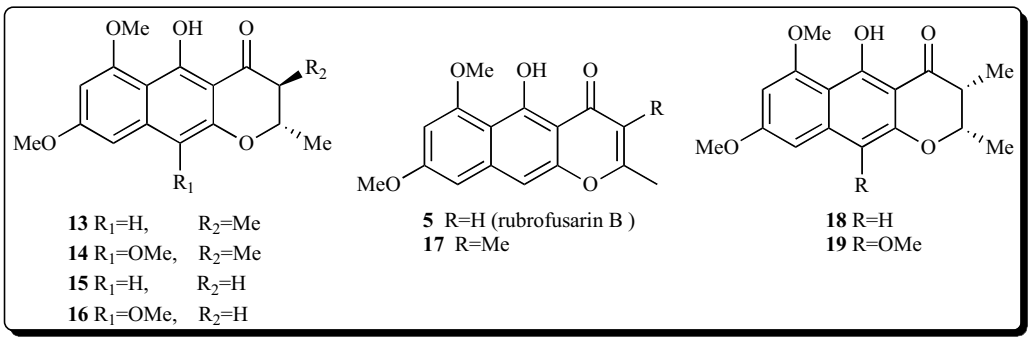


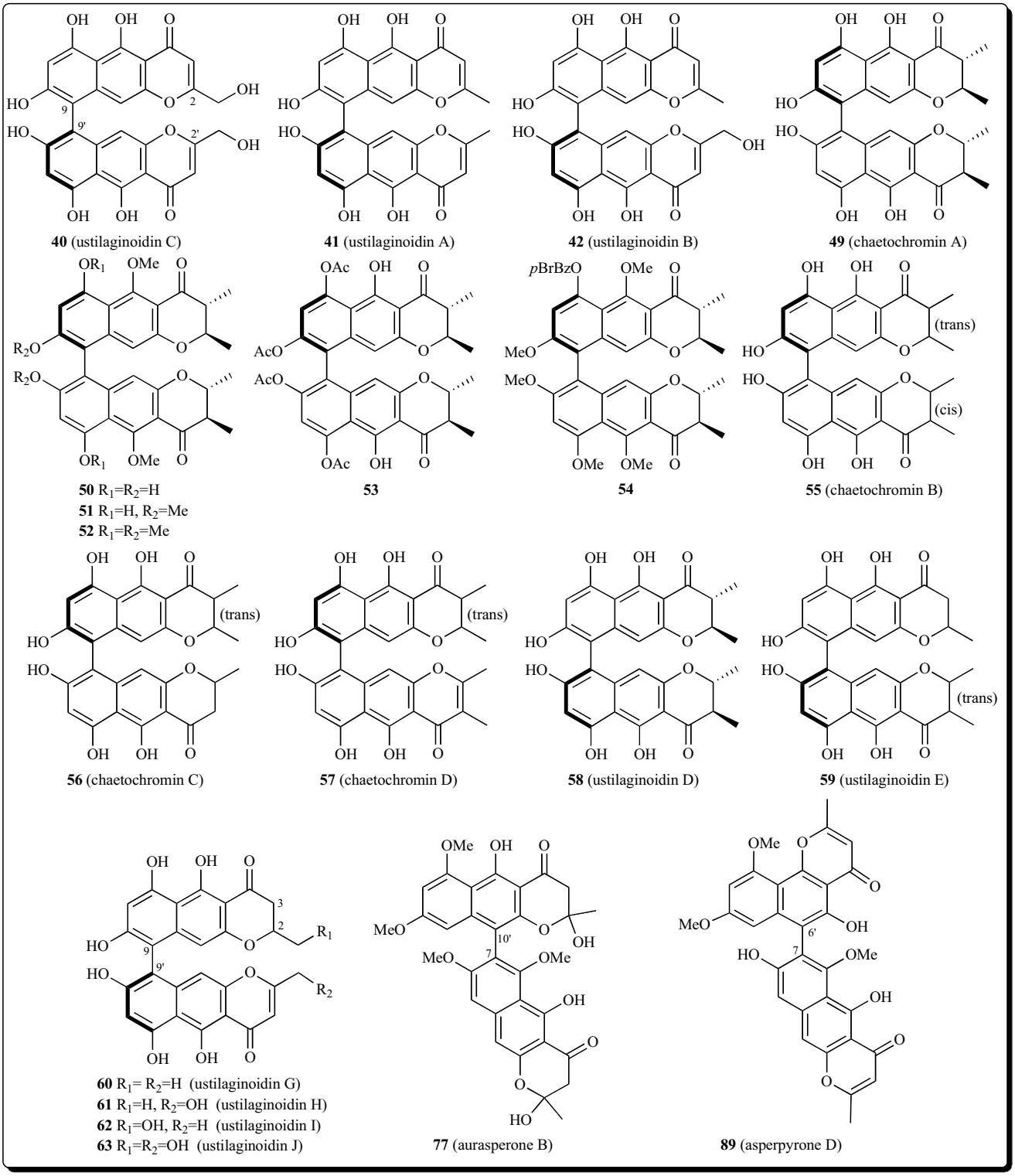


† This compound , isochaetochromin B_1 (**64**), is shown here with the same basic structure as chaetochromin B (**55**); however these are not identical compounds, they are diastereomeric and their absolute stereochemistries are unknown.

Chaetochromin A (**49**) was found to be active against HeLa cells at concentrations above 3.2μg/mL [67]. Subsequently a survey evaluating a large number of synthetic and natural LNGPs for activity against KB cancer cells was conducted. The compounds tested encompassed: twenty 9-9' DLNGPs *vis.* chaetochromin A-D (**49**, **55**, **56**, **57**) and five synthetic chaetochromin derivatives (**50**-**54**), ustilaginoidin A-E (**40**-**42**, **58**, **59**) & G-J (**60**-**63**), cephalochromin (**43**), and dihydroustilaginoidin A (**47**); three other DLNGPs nigerone (**84**), isonigerone (**85**), and aurasperone B (**77**); in addition to two monomeric LNGPs, *nor*-rubrofusarin (**3**) and rubrofusarin B (**5**).

The activity profile allowed preliminary structure activity relationships to be assembled. Strong activity was found only when the naphthopyrones were linked through the C9-9' positions forming symmetric bridged DLNGPs (Fig. **3**) with an unfunctionalised C2-methyl group and no substitution of the phenol groups. It was observed that increased phenolic substitution decreased activity, with the exception of a single acetate derivative **53** which was also strongly active. Given the facile nature of this acetate linkage under hydrolytic conditions it is reasonable to assume it is simply a prodrug of the parent compound. In these assays the 2,3-dihydro compounds showed more potent activity than the unsaturated compounds, which suggests in this case that a Michael acceptor capacity is not required for biological activity. The other DLNGPs and the monomeric rubrofusarin B (**5**) were inactive, whereas *nor*-rubrofusarin (**3**) displayed weak activity. Some of the active compounds, namely chaetochromin A-D, cephalochromin and ustilaginoidin A & G were further evaluated *in vivo* as antitumor agents in mice. While some of these compounds prolonged the life expectancy of the mice at low doses, they were considered too toxic at higher doses for further evaluation. The inhibition of

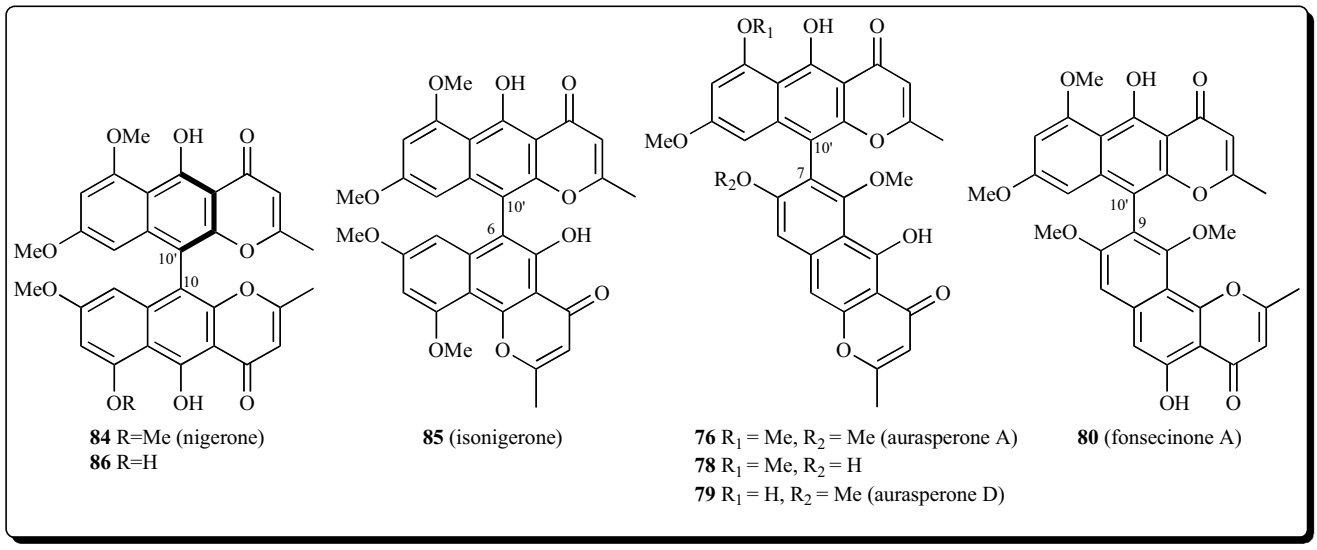




DNA, RNA and protein synthesis by ustilaginoidin A (**41**), chaetochromin A (**49**) and cephalochromin (**43**) was investigated to probe the mode of action. These results mirrored the cytotoxicity and were interpreted to suggest an unknown mode of action was responsible for the activity [29].

More recently the prenylated LNGPs, adenaflorins A (**32**) and B (**33**), have been found to show strong to moderate activity against MCF-7, H-460 and SF-268 human cancer cell lines [46]. Again, it was observed that the 2,3-dihydro compound, **32**, was more active than the unsaturated analogue.

Strong activity against SW1116 colon cancer cells is reportedly possessed by rubrofusarin B (**5**), with the DLNGPs aurasperone A (**76**) and fonsecinone A (**80**) showing moderate/low activity [36]. LNGPs have also shown drug resistance reversion properties with the semi-synthetic aurasperone derivative **78** and rubrofusarin B (**5**) showing the ability



84 R=Me (nigerone)
86 R=H

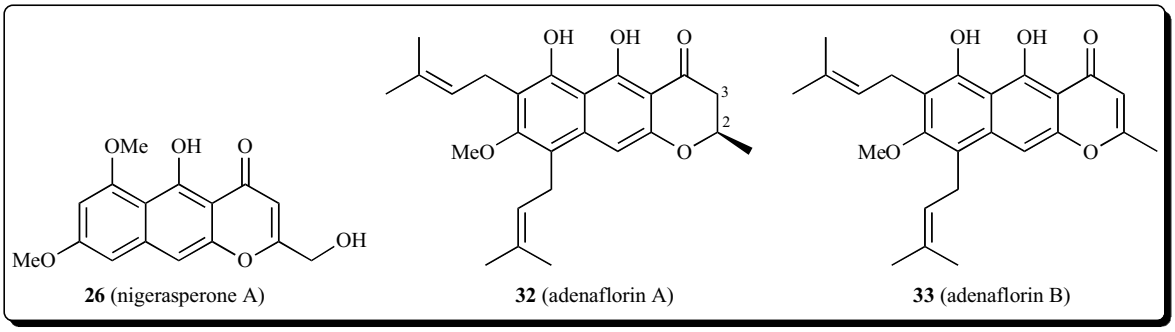
85 (isonigerone)

76 R_1 = Me, R_2 = Me (aurasperone A)
78 R_1 = Me, R_2 = H
79 R_1 = H, R_2 = Me (aurasperone D)

80 (fonsecinone A)

to reverse the resistance of human KB cancer cells to the drugs vincristine and mitomycin C [31, 98].

The toxicity of chaetochromin A (**49**) has been investigated on a number of occasions. In the first report on the



26 (nigerasperone A)

32 (adenaflorin A)

33 (adenaflorin B)

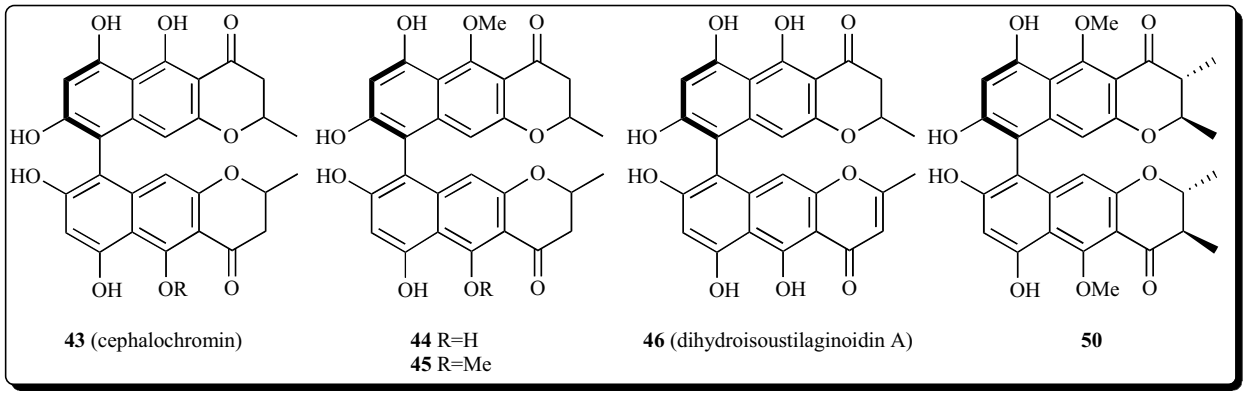
6. GENERAL TOXICITY (MAMMALIAN)

Due to the useful breadth of biological activities displayed by the LNGP structural class and their potential for clinical development, several toxicity studies have been conducted in animal models. Toxicological data that shed light on the LNGPs' mode of action would also be of use in a broader context given the occurrence of these compounds in fungi known to infect grains and foodstuffs consumed by humans and other animals. Studies looking at LNGP toxicity include specific biological evaluations (herein outlined) in addition to more general studies looking for the presence of these mycotoxins in various sources.

The first study of LNGP toxicity was conducted by Ghosal and colleagues who investigated the effects of rubrofusarin (**1**), aurasperone D (**79**) and a mixture of the total naphthopyrones from a strain of *Aspergillus niger*, on the central nervous systems of mice and rats. All three test samples were central nervous system depressants, though rubrofusarin was the weakest of these and did not lead to mortality at the higher doses reported. The LD_{50}s of aurasperone D and the mixture of naphthopyrones were 44 and 47 mg/kg [21]. It is notable that the monomeric LNGP was significantly less toxic than the dimeric compound.

isolation and structural elucidation of **49** it was shown to be acutely toxic to mice at 10 mg/kg when administered intraperitoneally. At the same time **49** was found to be inactive in the *Salmonella* microsome test [67]. Two major studies subsequently investigated the nature of the biological activity. In the 1980s Ohtsubo and colleagues initiated a number of investigations into the toxicity of chaetochromin A, while in the 1990s Natori and colleagues, the original discoverers of chaetochromin A, along with associated researchers conducted a series of toxicological and anticancer investigations into **49** and related compounds.

Early studies by Ohtsubo and colleagues showed chaetochromin A was orally toxic to mice at around 400 mg/kg, and at various concentration levels it induced injuries in a number of organs including the liver, spleen, bone marrow and lymph nodes [64]. Similar results were found when mice were fed rice infected with the whole producing organism, *Chaetomium gracile* [65]. The same authors later noted that various organs were damaged in mice fed **49** at 30 mg/kg but the deleterious effect was not observed at a regime of 10 mg/kg [59]. Additionally they recorded that when pregnant mice were fed a diet containing **49** at 30 mg/kg, an increase in fetal malformation and death was observed, indicating that the DLNGP is teratogenic [58, 59]. Subsequent studies on



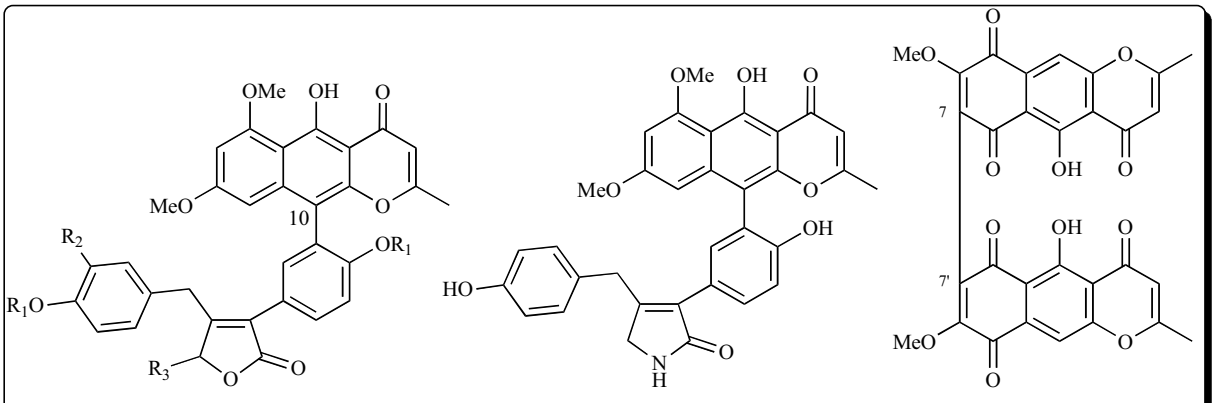
43 (cephalochromin) **44** R=H **45** R=Me **46** (dihydroisoustilaginoidin A) **50**

the oral toxicity of chaetochromin A in mice, revealed more detailed information regarding the effects of various doses on organ damage, and gave an estimated LD_{50} of 152 mg/kg [66].

Natori and colleagues have also investigated DLNGP toxicity. Initially chaetochromin A (**49**), chaetochromin D (**57**) and ustilaginoidin A (**41**) were tested *in vitro* as inhibitors of differentiation of embryonic mouse cells, and all three compounds were shown to be teratogenic [49]. To further understand the origin and mode of toxicity in chaetochromin A and related DLNGPs, their *in vitro* effect on the mitochondria of rat heart and liver cells was examined. Chaetochromin A, ustilaginoidin A and cephalochromin (**43**) were found to disrupt ATP synthesis in mitochondria by interfering with oxidative phosphorylation [60]. Mitochondrial swelling was also induced by chaetochromin A (**49**) but this was eliminated in the presence of magnesium ions [50]. Further studies examined the effect of pH and various alkali metal ions on mitochondrial swelling induced by chaetochromin A [99]. Later the activity of chaetochromin D (**57**) on mitochondrial swelling and ATP synthesis was examined, and perhaps unsurprisingly chaetochromin D was found to exhibit similar activity to that observed for chaetochromin A [63].

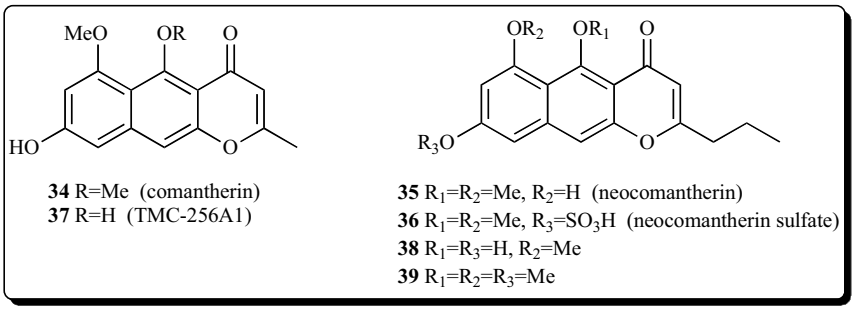
The interaction of chaetochromin A (**49**) with bovine serum albumin was studied spectrophotometrically to provide information regarding protein binding. The results suggested that the anion of chaetochromin A bound with an anion-binding site in the protein [61]. These experiments were elaborated upon when it was shown that chaetochromin A was fluorescent under hydrophobic conditions. Under normal aqueous conditions it was not fluorescent but fluorescence emerged in the presence of bovine serum albumin or with a cationic detergent. Thus it was concluded that chaetochromin A might bind with cationic residues in a hydrophobic protein pocket [62]. Further, magnesium ions altered the spectrum of anionic chaetochromin A, which suggested a chelated complex was formed, providing a possible explanation for the earlier observed mitigation of mitochondrial swelling upon addition of magnesium ions [61]. Around the same time a different group of workers found cephalochromin (**43**) was a potent inhibitor of calmodulin-sensitive phosphodiesterase [52].

There are a number of miscellaneous studies concerning LNGP toxicity. One of these mentions the toxicity of specific compounds yet does not give details about how their toxicity was evaluated. Gorst-Allman and colleagues noted that the LNGPs they obtained from *A. niger* **84**, **85**, **86**, and **5**



8 R_1=R_2=H, R_3=OH (carbonarin A)
10 R_1=H, R_2=OMe, R_3=OH (carbonarin F)
11 R_1=R_3=H, R_2=OMe (carbonarin G)
12 R_1=R_2=R_3=H (carbonarin H)

9 (carbonarin C) **147** (aurofusarin)



34 R=Me (comantherin)
37 R=H (TMC-256A1)

35 $R_1=R_2=Me$, $R_2=H$ (neocomantherin)
36 $R_1=R_2=Me$, $R_3=SO_3H$ (neocomantherin sulfate)
38 $R_1=R_3=H$, $R_2=Me$
39 $R_1=R_2=R_3=Me$

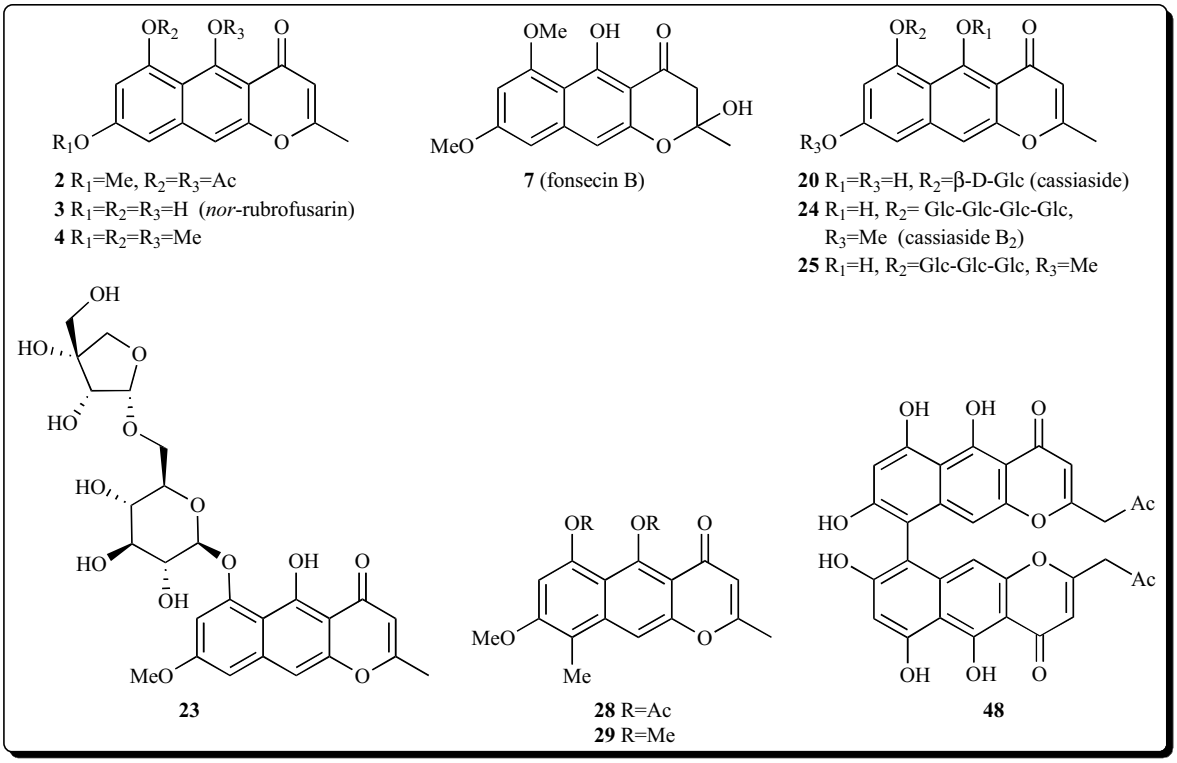
"do not contribute to the toxigenicity of the fungal culture" yet did not substantiate this claim [30]. The other two miscellaneous studies cannot be associated with specific compounds; in the first, Ehrlich and co-workers injected mice with various doses of a crude mixture containing *"more than 18 components"* including eight NGPs and found the mixture was acutely toxic [10]. The other non-specific reference is from a paper on poisoning caused by some compounds in *Ustilaginoidea virens* which states *"ustilaginoidins… were not the causative compounds as the phytotoxin or mycotoxin"* [100].

Recently chaetochromin A (**49**), dihydro-isoustilaginoidin A (**46**), and cephalochromin (**43**) were found to be inhibitors of nitric oxide production in a macrophage-like cell line. The cephalochromin 5-methyl ether derivative, **44** showed a significant reduction in this activity, whereas the 5,5'-dimethyl derivative **45** was devoid of activity entirely. This mirrored earlier observed structure activity relationships *vide supra*, where a naked 5-OH was correlated with anticancer activity, whereas chaetochromin A 5,5-dimethyl ether (**50**) showed a substantial reduction in the activity. It was proposed that these results suggested the C-3-methyl group enhanced activity, however, no mechanism to explain this was proposed. Further experiments with the active compounds showed inhibition of mRNA expression was responsible for the activity, rather than inhibition of the nitric oxide synthase [53].

7. LNGP ENTOMOLOGICAL BIOTESTING

Interest in entomologically active compounds is driven by their application in the treatment of parasite infestations in both humans and animals. The LNGP containing carbonarins (**8-12**) all possessed varying degrees of insecticidal activity in a variety of assays. Three additional carbonarins that do not contain LNGP moieties were also reported all of which possessed some activity, indicating that the LNGP may not be vital for insecticidal activity but may enhance it [37].

Rudman and Gay tested rubrofusarin (**1**) for antitermitic activity in 1963, finding no statistically significant deterrent activity or toxicity towards *Cryptotermes brevis*. Interestingly, aurofusarin (**147**) which is not a LNGP but instead a



2 $R_1=Me$, $R_2=R_3=Ac$
3 $R_1=R_2=R_3=H$ (*nor*-rubrofusarin)
4 $R_1=R_2=R_3=Me$

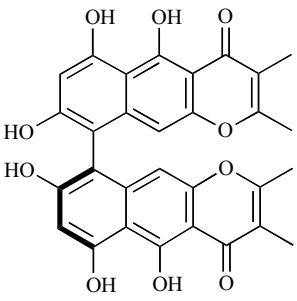
7 (fonsecin B)

20 $R_1=R_3=H$, $R_2=\beta$-D-Glc (cassiaside)
24 $R_1=H$, $R_2=$ Glc-Glc-Glc-Glc, $R_3=Me$ (cassiaside B_2)
25 $R_1=H$, $R_2=$Glc-Glc-Glc, $R_3=Me$

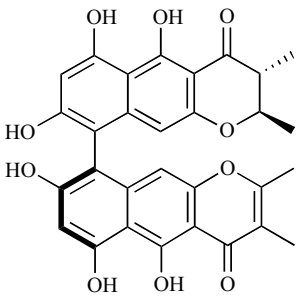
23

28 R=Ac
29 R=Me

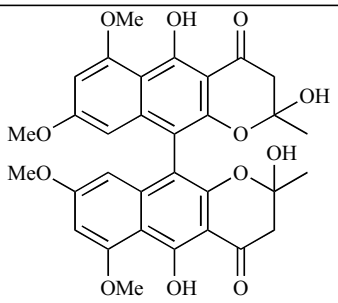
48



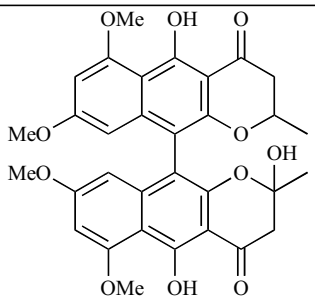
74 (ustilaginoidin K)



75 (ustilaginoidin L)



91 (10,10'-bifonsecin B)



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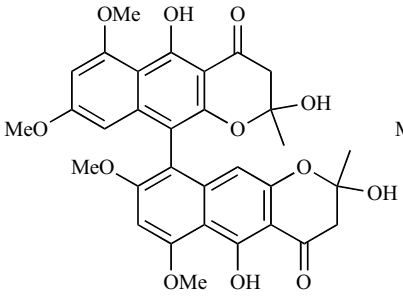

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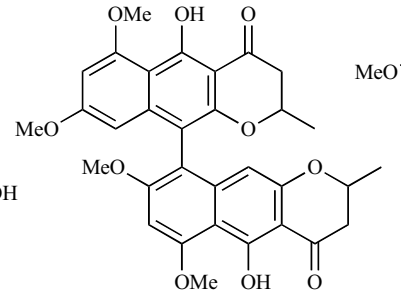

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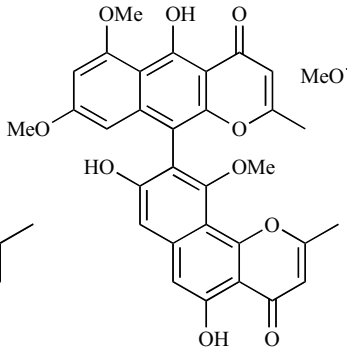

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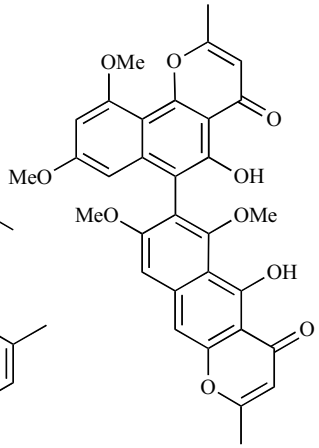

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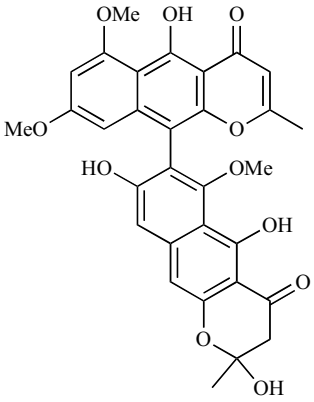

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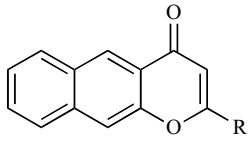

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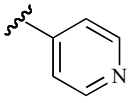

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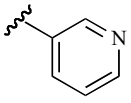

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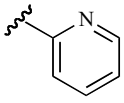

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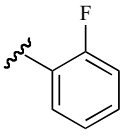

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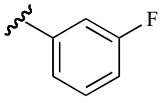

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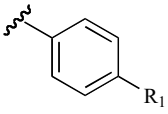
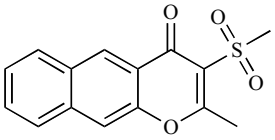


101 R_1=F
102 R_1=Br
103 R_1=OMe



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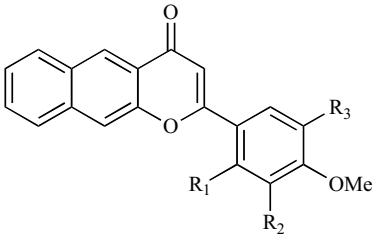
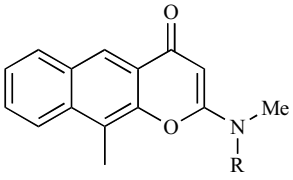
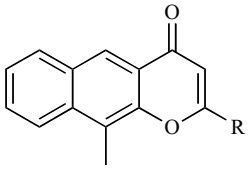


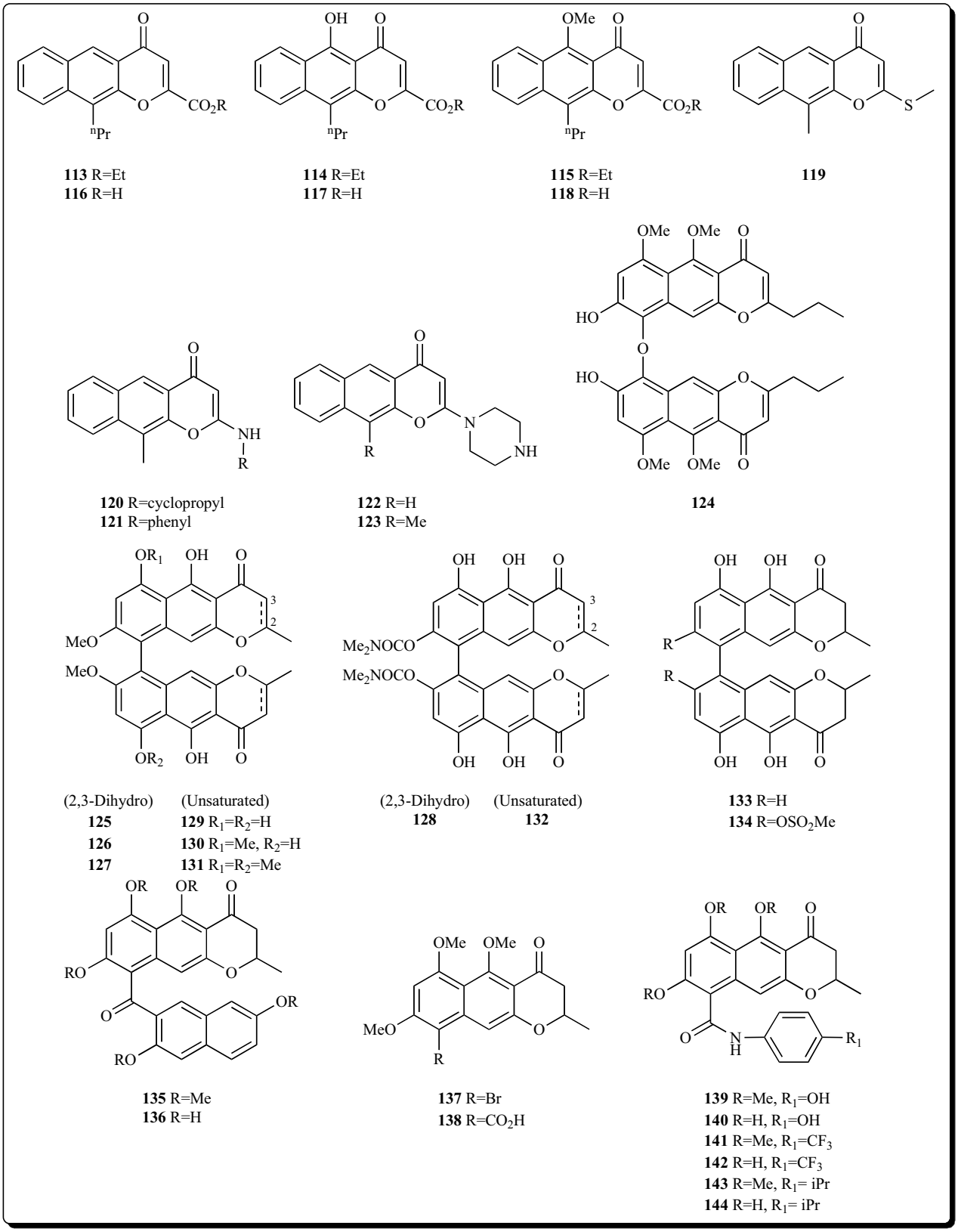
105 R_1=R_2=H, R_3=OMe
106 R_1=R_2=H, R_3=OH
107 R_1=H, R_2=R_3=OMe



108 R=Me
109 R=Et



110 R=NEt_2
111 R=*N*-pyrrolidine
112 R=*N*-morpholine



naphthoquinone and can be thought of as a C7-C7' dimer of an oxidised rubrofusarin core, proved to be an effective deterrent in this assay [101].

8. MISCELLANEOUS BIOASSAYS

The ability to provide commentary on the miscellaneous bioassays listed in Table **2** and cross referenced to column 8

of Table **1** is beyond the scope of this review which is designed to introduce the reader to the breadth and potential of this class of compound. The structures of the bioactive components within Table **1** are shown and the interested reader is directed to the associated references. For example, examination of the fish antifeedant assay within Table **2** (Assay 17), would reveal the LNGPs isolated from the marine organism *Comantheria perplexa*, comantherin (**34**) and neocomantherin (**35**) which showed strong antifeedant activity towards a variety of fish species. A similar level of activity is found with the semi-synthetic neocomantherin sulfate (**36**) [47].

SUMMARY

We have attempted to give coverage to all LNGPs that have received biological testing to April 2008 with an emphasis on those compounds that possess activities of potential interest within a clinical environment. The review represents the first of its kind, focusing on the linear naphtho-γ-pyrone structural motif and should serve to highlight the remarkable diversity of biological activities displayed and the therapeutic potential presented by this evolving group of compounds. While the notable studies and activities have been presented above to position the researcher within the field, the reader is directed toward Table 1 and the references therein for in depth detail.

We are acutely aware that little mention has been made regarding synthetic efforts directed toward the development of the LNGP skeleton. However, a manuscript reviewing synthetic approaches toward this emerging class of compound, complementing the material presented here, is in preparation and will appear in a separate publication.

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