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REVIEW

Progress in the studies on tryptanthrin, an alkaloid of history

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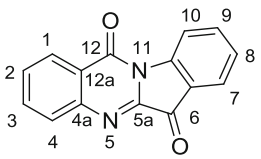
Abstract Tryptanthrin, an indoloquinazoline alkaloid, was first obtained by sublimation of natural indigo and later isolated from the culture of fungus *Candida lipolytica* and a variety of other natural sources. Tryptanthrin showed a variety of intriguing biological properties such as antibacterial, antifungal, antiprotozoal, and antiparasitic activities, inhibitory activities against COX-2, 5-LOX, NO synthase and PGE(2) expression, as well as cytotoxic and antitumor activities. Present review covers recent studies on the natural sources, biological activities and mechanisms of their actions, synthesis, structure–activity relationship, and metabolism of tryptanthrin.

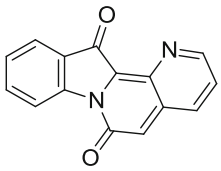
Keywords Tryptanthrin · Alkaloid · Indoloquinazolinone

Introduction

The history of tryptanthrin (**1a**, 6,12-dihydro-6,12-dioxoindolo[2,1-*b*]quinazoline) began as early as in 1822 (Siedel 1822). In 1879 Sommargua described the sublimation of natural indigo under reduced pressure to give yellow needles with a molecular formula of $C_{15}H_8N_2O_2$ (m.p. 258–259 °C) (von Sommaruga 1879). Several research groups additionally reported the same golden-yellow crystals from commercial as well synthetic indigos (O'Neill 1892; Bloxam 1915) and indigotin as well (Perkin, 1906). German patent reported the same yellow compound as an oxidation product of indigo by $KMnO_4$ (German Patent 1913). Friedländer and Roschdestwensky not only prepared the compound by air oxidation of indigo at high temperature and designated it as anhydro-α-isatinanthranilide with a structure but also prepared it chemically from anthranilic acid and its equivalent (Friedländer and Roschdestwensky 1915).



1a



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Tryptanthrin was also isolated from the culture of the yeast *Candida lipolytica*, grown in an artificial media containing high concentration of tryptophan, hence it was designated as tryptanthrin in 1971 (Schindler and Zähner 1971). It should be noted that Sen et al. (1974) isolated yellow needles (m.p. 265–266 °C, m/e 248) from petroleum extract of dried and powdered fruit of *Couroupita guaianensis* Abul in 1974 and determined its structure as **2** with a trivial name couroupitine A (Sen et al. 1974). However the structure of couroupitine A was corrected later by Bergman et al. (1977) as the structure **1a** originally proposed.

The structure of tryptanthrin is so unique that the preliminary chemical and spectroscopic (UV, IR, and NMR) (Jahng 2012)[1] data are not sufficiently definite enough to characterize its structure. The exact structure, thus, remained uncertain until X-ray crystallography confirmed

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[1] Spectral data for tryptanthrin were collected as follows: IR(KBr) υ 1725, 1688, 1610, 1519, 1550, 1350, 1310, 760 cm^{-1}; UV (EtOH) λ_{max} (ε) 225 (4.46), 251 (4.68), 280–420 (4.04) nm; MS *m/z* (rel. intensity) 248 (M, 100), 220 (45), 192 (30), 102 (15). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 182.6 (C6), 158.1 (C12), 146.6 (C4a), 146.3 (C10a), 144.3 (C5a), 138.3 (C9), 135.1 (C3), 130.7 (C4), 130.2 (C2), 127.5 (C1), 127.2 (C8), 125.4 (C7), 121.9 (C6a), 118.0 (C10).

Table 1 The least-square planes, in terms of monoclinic co-ordinates, with distances (Å) of the atoms from the plane through the pyrimidine ring N(11), N(5), C(12), C(5a)–C(12a) (Fedeli and Mazza 1974)

$0.9347x + 0.3556y - 0.3038z + 0.3264 = 0$					
Deviations					
N(11)	−0.017	C(4a)	0.005	N(5)	−0.015
C(12a)	−0.014	C(12)	0.002	O	0.090
C(5a)	0.007				

the present structure (Brufani et al. 1971; Fedeli and Mazza 1974)[2]. An X-ray crystal structure of tryptanthrin showed that its main structural feature is a nearly planar arrangement within experimental error. However, there are small but significant departures from the planarity, especially for the 'O' and probably 'C(12)' of carbonyl on the pyrimidine ring (see Table 1).

All the proton resonances have been assigned and are summarized in Table 2 (Jarrah and Thaller 1980). H10 lies in the bay region and thus is the most down-field (δ 8.67) shifted one due to the deshielding effect of oxygen at C(12) carbonyl. Such deshielding effect also leads H1 and H7 to resonate at δ 8.48 and 7.96, respectively, while the lone pair of N5 similarly affects the chemical shift of H4.

Although a plenty of studies for tryptanthrin resulted in a couple of reviews, these were only focused on the synthesis of the tryptanthrin core and reactions of it (Witt and Bergman 2003; Wang et al. 2007; Tucker and Grundt 2012). Present review covers the occurrences, physicochemical and biological properties, synthesis and reactions as well as structure and activity relationships.

Occurrence and derivatives

Tryptanthrin was additionally isolated from fungi such as *Schizophyllum commune* (Hosoe et al. 1999, 2000) and *Leucopaxillus cerealis* (Jarrah and Thaller 1980), and higher plants such as *Couroupita guaianensis* Abul. (the cannon ball tree) (Sen et al. 1974; Bergman et al. 1977, 1985), *Strobilanthes cusia* (assam indigo) (Honda and Tabata 1979), *Polygonum tinctorium* Lour. (Japanese and Chinese Indigo) (Honda et al. 1980), *Isatis indigotica* (wood) (Li 1987; Li et al. 1983, 2000)[3], known as 'Qing Dai (leaves)' and 'Ban Lan Gen (roots)' in Chinese, 'Sentai' in Japanese, and 'Cheongdae' in Korean traditional medicine, *Isatis tinctoria* (Honda et al. 1980; Seifert and Unger 1994; Danz et al. 2001, 2002a) known as Chinese woad, *Wrightia tinctoria* (George et al. 1996; Muruganandam and Bhattacharya 2000), two Calanche species including *C. discolor and C. liukiuensis* (Yoshikawa et al. 1998; Murakami et al. 2001), *Phaius mishmensis* (Jao et al. 2008), *Cissus sucyoides* (Xu et al. 2009), and *Baphicacanthus cusia* (Liu et al. 2009). It is somewhat intriguing that tryptanthrin has also been isolated from the urine of Asian elephant (*Elephas maximus* (Rasmussen et al. 1993).

The derivatives of tryptanthrin, isolated from the natural sources, are summarized in Fig. 1. Methylisatoid (Baeyer and Oekonomides 1882) and candidine (Laatsch and Leudwig-Köhn 1986) were chemically prepared long before their isolation from natural sources. Methylisatoid is a compound of controversy with a long history (vide infra). On the other hand, candidine (also known as qingdainone) was isolated by Fielder from the culture of *Candida lipolytica* as an unidentified violet compound with the molecular formula $C_{23}H_{13}N_3O_2$ (Fielder 1974)' of which the structure was later determined and named candidine by Bergman et al. (1985a; 1985b) Candidine was also additionally isolated from the higher plants such as *Isatica indigotica* (Zou and Huang 1985) and *Baphicacanthus cusia* (Wu et al. 1997). Ophiuroidine (4,8,9-trihydroxy tryptanthrin) was recently isolated from the marine invertebrate Carabian britterl star *Ophiocoma riisei* as the first hydroxylated tryptanthrin (Utkina and Denisenko 2007). An additional hydroxylated tryptanthrin, phaitanthrin C, and tryptanthrin-related compounds phaitanthrin A, B, D and E were also isolated from *Phaius mishmensis* along with methylisatoid and candidine (Jao et al. 2008).

Biological activities and reaction mechanisms

Although a variety of biological properties of tryptanthrin-containing herbs themselves and their extracts have been studied, this review will focus on tryptanthrin and its derivatives. Tryptanthrin showed strong inhibitory activities against pathogenic microorganisms such as *Bacillus subtilis* (MIC's of 3.1–6.3 μg/mL) (Schindler and Zähner 1971; Honda et al. 1979; Okunade and Elvin-Lewis 2009), *Escherlichia coli* (Bandekar et al. 2010), *Mycobacterium tuberculosis* (MIC = 10 μg/mL) (Baker and Mitscher 1995; Mitscher and Baker 1998a, b), and *Helicobacter pylori* (2.5 μg/mL) (Hashimoto et al. 1999; Kataoka et al. 2001), and antifungal activity against *Trichophyton*, *Microsporum*, and *Epidermophyron* species at the level of MIC = 5 μg/mL (Honda and Tabata 1979; Honda et al. 1980; Li et al. 1983). The antifungal activity of tryptanthrin is comparable to that of clinically using griseofulvin against *T. mentagrophytes.*

[2] In which crystal data of tryptanthrin were given: $C_{15}H_8N_2O_2$, MW = 248.2; monoclinic, $a = 7.46$ (6), $b = 7.66$ (6), $c = 20.78$ (16) Å, $\beta = 109.0$ (5)°, V = 1122.7 Å ^{3}; F (000) = 512; Space group $P2_1/c$ (C^5_{2h}, No. 14) from systematic absences; Z = 4, D = 1.47 g cm^{-3}.

[3] *Chem Abstr.*, 134, 128466(2001), Wikipedia encyclopedia has claimed that *Isatis indigotica* is a wrong expression of *Isatis tinctoria*: http://www.wikipedia.org.

Table 2 Data from 300 MHz ^{1}H NMR spectra of tryptanthrin in $CDCl_3$ (Jarrah and Thaller 1980)

	H1	H2	H3	H4	H7	H8	H9	H10
δ	8.48 (ddd)	7.72 (ddd)	7.90 (ddd)	8.08 (ddd)	7.96 (ddd)	7.83 (td)	7.83 (td)	8.67 (dd)
J^a	8.0,1.5,0.8	8.0, 7.5,1.5	8.0,7.5,1.5	8.0,1.0,0.5	8.0,1.5,0.5	8.0,1.5	8.0,1.5	8.0,1.5

[a] Coupling constant shown in Hz and in order of ^{3}J, ^{4}J, and ^{5}J, respectively

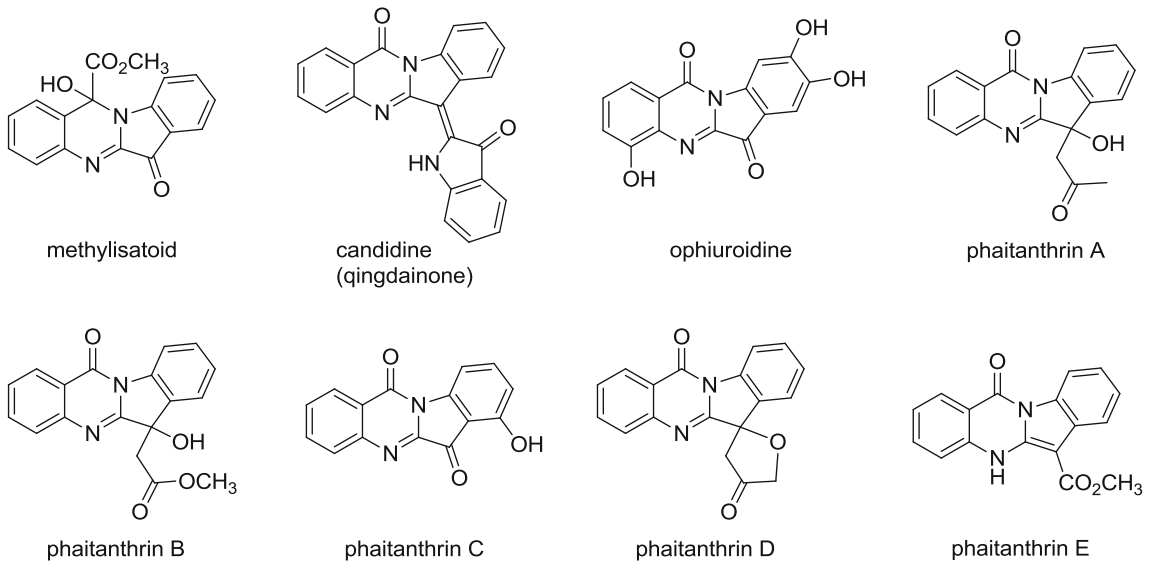


Fig. 1 Tryptanthrin derivatives isolated from natural sources

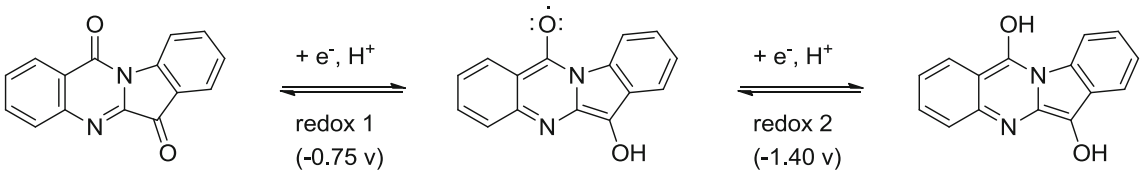
Its antiprotozoal activities against *Leishmania donovani* (IC_{50} = 600 ng/mL) (Bhattacharjee et al. 2002) and *Plasmodium falciparum* (<100 ng/mL) (Pitzer et al. 2003; Bhattacharjee et al. 2004), and anti-parasitic activity against *Trypanosoma brucei* (Scovill et al. 2002) were also strong enough to warrant further studies for its development as an anti-leishmanial agent (Bhattacharjee et al. 2002). The inhibitory activities of tryptanthrin against COX-2 (IC_{50} = 64 nM) (Danz et al. 2001, 2002b; Hamburger and Danz 2000), 5-LOX (IC_{50} = 0.15 μM) (Hamburger 2002; Danz et al. 2002a; Oberthür et al. 2005), NO synthase and prostaglandin E(2) expression at the cellular level (Ishihara et al. 2000) opened a vista for a possible lead for anti-inflammatory agents. In addition, the inhibitory activities against hepatocyte growth factor in human fibroblasts (Motoki et al. 2005) and against the multidrug resistance gene MDR1 in breast cancer cells (Yu et al. 2007, 2009) as well as the cytotoxicity against selected human cancer cell lines (IC_{50} = 10 μM for HT-1376) (Zou and Huang 1985; Hosoe et al. 1999; Shaema et al. 2002; Jao et al. 2008; Camargo et al. 2009; Yu et al. 2007; Liang et al. 2012) and the antitumor activity (Kimoto et al. 2001; Koya-Miyata et al. 2001; Chan et al. 2009) of tryptanthrin were also studied.

However, studies on the mechanism of biological activities are limited to include hemin and hemozoin binding as a factor for antimalarial activity (Dorn et al. 1998; Hicks et al. 2005), DNA intercalation (~10 drug/kbp at 100 μg/mL) for antibacterial activity against *E. coli* (Bandekar et al. 2010), and down-regulation of MRD1 gene expression in doxorubicin resistance to human breast cancer (Yu et al. 2007, 2009), and modulatory action on murine myeloid leukemia cells (Chan et al., 2009).

Although systematic synthesis and structure–activity relationship studies for anti-tubercular (Mitscher and Baker 1998a), COX inhibitory (Hamburger and Danz 2000), and cytotoxic activity (Zou and Huang 1985; Sharma et al. 2002; Camargo et al. 2009; Liang et al. 2012), have led several promising candidates for further development, none of them has yet been successfully launched to the clinics (vide infra).

In addition to such intriguing biological properties, tryptanthrin can also transport electrons, thus having potential as a photoelectronic photoreceptor (Sugai and Saito 1997; Sugai et al. 1997). The origin of such a property stems from the electron-accepting ability of tryptanthrin. Tryptanthrin shows two reversible waves with cathodic and anodic peaks, separated by approximately 60 mV, indicating two one-electron transfers. The more facile (less negative) reduction potential at −0.75 V covers an electron transfer to the carbonyl oxygen on the more strained five-membered ring,

while the more negative potential at –1.40 V covers an electron transfer to that of the less strained six-membered ring. The electron-accepting ability of the carbonyl atoms seems to be crucial for the antileishmanial activity of tryptanthrin (Bhattacharjee et al. 2002).



Total synthesis and other chemical studies

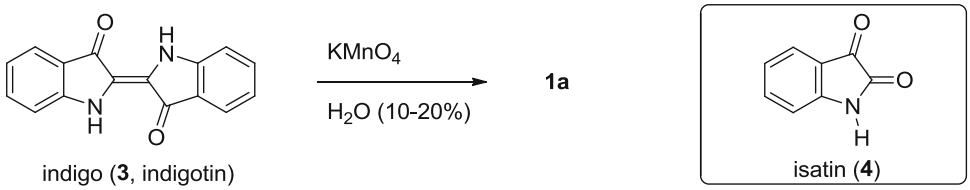
Synthesis

There might be three general approaches for the synthesis of tryptanthrin: oxidative coupling of isatin, indigo, and methylisatoid; the construction of quinzolinone ring at the final key step, and the construction of indol ring at the final step. However, many of the reactions would not allow such simple categorization; the present review was, thus, written in the chronological order on the basis of new synthetic approaches.

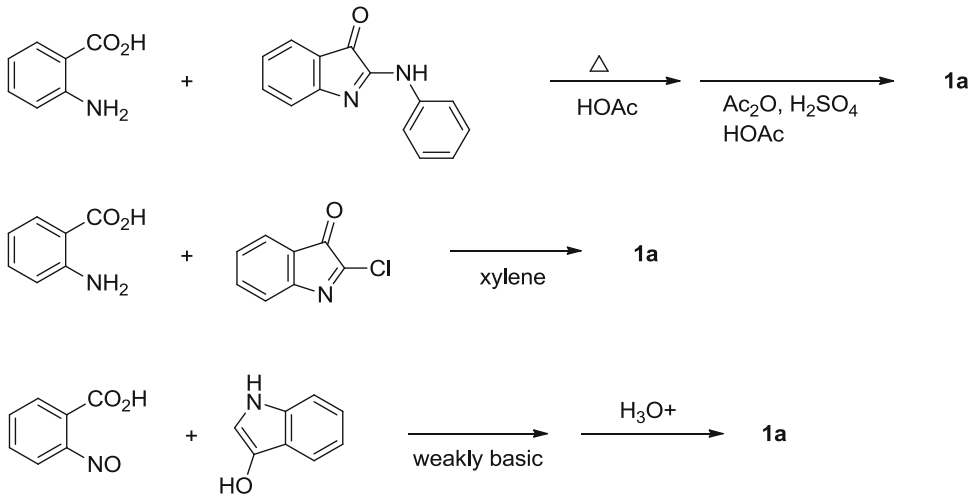
The early preparation studies on tryptanthrin included the chemistry of indigo (**3**, indigotine) and methylisatoid. Meister et al (1913) isolated the same yellow compound as an oxidation product of indigo by $KMnO_4$ (German Patent belongs to Farbwerke vonmals Meister 1913). The same result was also obtained from isatin (**4**).

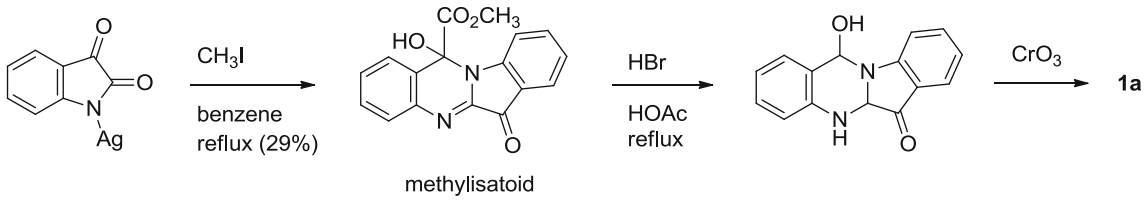
Friedländer and Roschdestwensky prepared anhydro-α-isatinanthranilide three different ways from α-isatinanilide and anthranilic acid, isatin chloride and anthranilic acid, and *O*-nitrosobenzoic acid and indoxyl (Friedländer and Roschdestwensky 1915) (Scheme 1). These synthetic approaches opened a new vista for the preparation of tryptanthrin chemically.

Heller and Benade reported tryptanthrin by CrO_3 oxidation of dihydrotryptanthrin which was prepared by heating methylisatoid with HOAc-HBr (Heller 1919; Heller and Benade 1922). However, the exact structure of methylisatoid and related alkylisatoids had been a long unfruitful controversy between Heller and Hantzsch. Heller favored an *O*-methylisatin-isatin complex structure for methylisatoid (Heller and Benade 1907, 1922), while Hantzsch preferred one of two monomeric structures (Hantzsch 1921, 1925). Such controversy was ended by Cox et al. who excluded Heller's structure by X-ray crystallography structure determination (Cox et al. 1936). However, Hantzsch's structures also turned out to be ill-characterized when Cornforth proved the present structure by three experiments including decomposition and synthesis (Cornforth 1976).



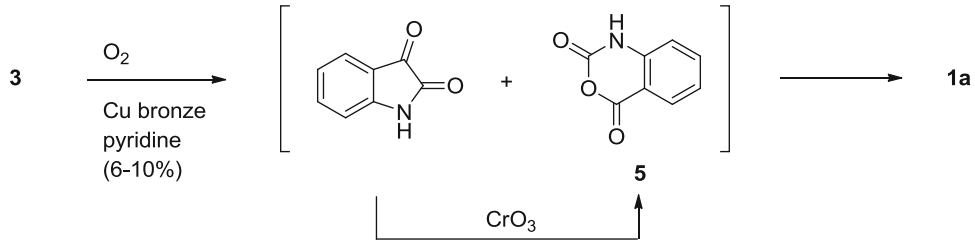
Scheme 1 Three synthetic methods for anhydro-α-isatinanthranilate (tryptanthrin) by Friedlaender and Roschdestwensky



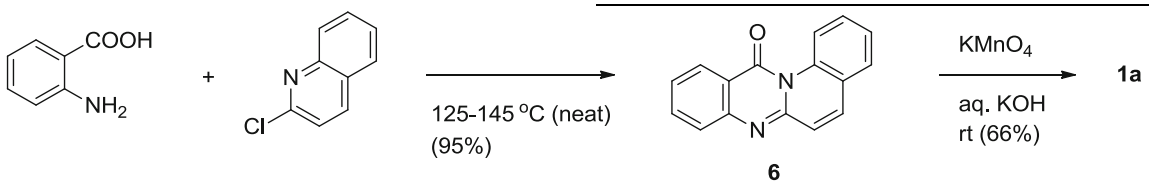


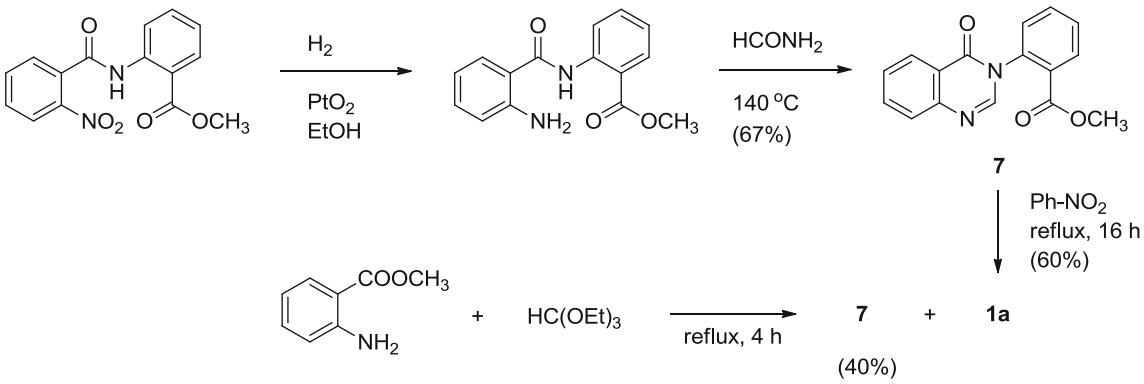
Air oxidation of indigo blue (also called indigotin) in the presence of Cu and pyridine also afforded tryptanthrin (Machemer 1930), which can also be achieved from indigo and isatin in the presence of $Cu(OAc)_2$ (Heller and Barthel 1936). Such a method has been re-examined using ozone as the oxidant to provide isatin, isatoic anhydride, and tryptanthrin. Isatin formed as a primary intermediate would then undergo further oxidation to isatic anhydride (**5**) (Matsui et al. 1982), which would be condensed to isatin to form tryptanthrin. Later, oxidation of isatin by oxidizing agent such as CrO_3 to isatoic anhydride was proven to be effective (Geckeler and Metz 1979).

Condensation of methyl 2-(2′-aminobenzamido)benzoate and formamide afforded 3,4-dihydro-3-(2-methoxycarbonylphenyl)-4-oxoquinazoline (**7**), which was then cyclized by heating at 140 °C to give tryptanthrin in 60 % yield. Similarly reaction of methyl anthranilate with ethyl orthoformate afforded **7** in 40 % yield and tryptanthrin, where the yield of trypanthrin was not given (Butler et al. 1960).



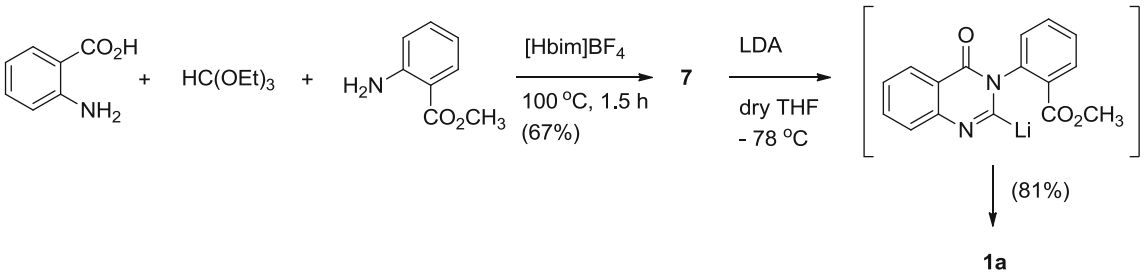
Zeide and Chelintsev employed a nucleophilic substitution reaction of 2-chloroquinoline with anthranilic acid followed by cyclization to form 12*H*-quino[2,1-*b*]quinazolin-12-one (**6**) (the reverse process cannot be excluded) and subsequent oxidation of **6** by $KMnO_4$-mediated oxidative ring contraction to afford tryptanthrin (Zeide and Chelintsev 1937).



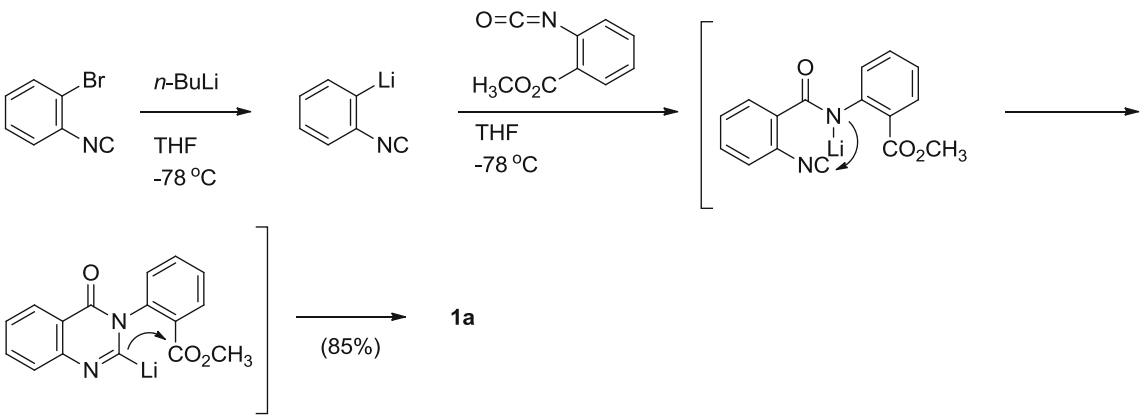


This method has been modified recently. Three-component reaction of anthranilic acid, methyl anthranilate, and ethyl orthoformate in the presence of liquid crystal, [Hbim]BF_4, resulted in **7** in 67 % yield. The C2-H of quinazolin-4(3*H*)-one is so acidic enough to be lithiated by *n*-BuLi at room temperature, which enables to cyclize to tryptanthrin in 81 % yield (Potewar et al. 2008).

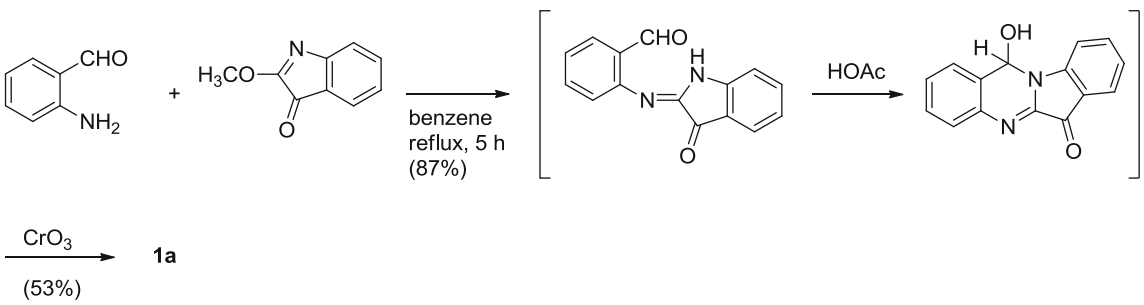
The reaction of *N*-sodioisatin with 2-nitrobenzoyl chloride gave 1-(2′-nitrobenzoyl)isatin (**8**), in which the nitro group could be readily reduced by a conventional reduction method, such as refluxing with $SnCl_4$ in conc. HCl to afford tryptanthrin in 67 % yield (Kikumoto and Kobayashi 1966; Son et al. 2003).

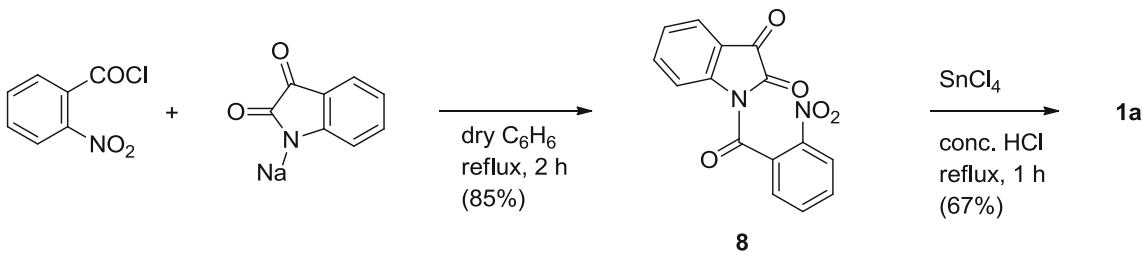


The intermediate, 2-lithiated 3-(2′-methoxycarbonylphenyl)quinazolin-4(3*H*)-one, could be directly generated from (2-isocyanophenyl)lithium, which was the reacted with methyl 2-isocyanatobenzoate to afford tryptanthrin in 85 % yield (Lygin and de Meijere 2009).



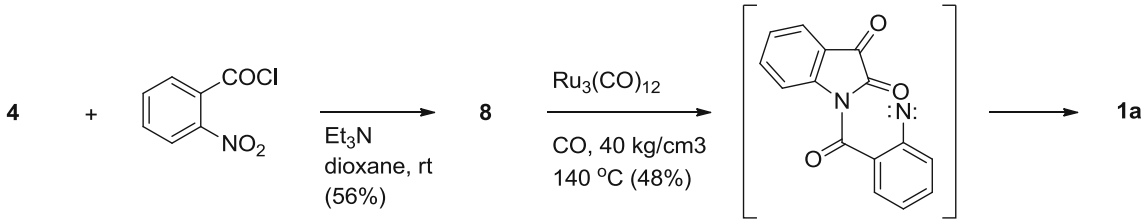
The preparation method of Bird in 1963 involved Schiff base formation of *O*-methylisatin with 2-aminobenzaldehyde, which then underwent acid-catalyzed cyclization followed by CrO_3 oxidation to give tryptanthrin (Bird 1963).



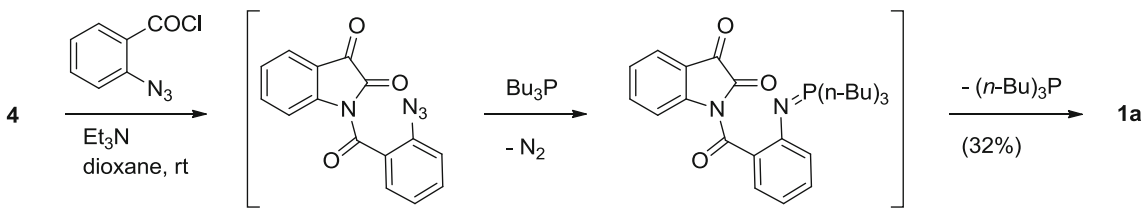


In addition, a reaction of **8** in the presence of $Ru_3(CO)_{12}$ and CO (*g*) under pressure (40 kg/cm^3) provided tryptanthrin in fairly good yield (Akazome et al. 1993). This procedure involves well-known deoxygenative reduction of a nitro group by carbon monoxide to an active nitrene intermediate (Nugent and Mayer 1988)[4]. The resulting nucleophilic nitrene then attacks the C2 carbonyl group of isatin to form the desired tryptanthrin.

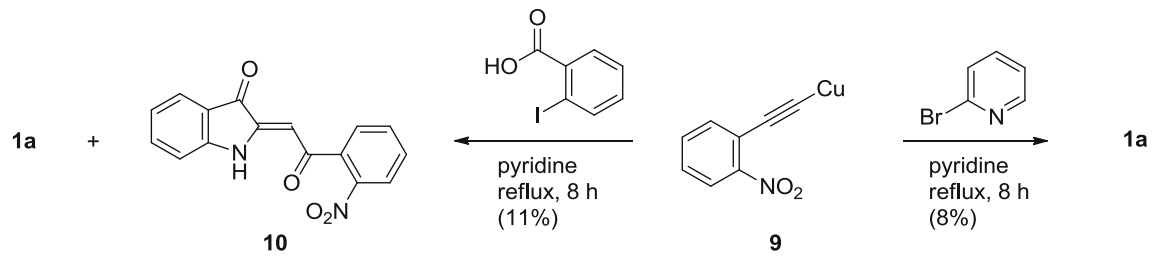
Bond and Hooper reported a reaction of copper 2-nitrophenylacetylide (**9**) with 2-iodobenzoic acid in pyridine to form tryptanthrin in 11 % yield, along with indogenide (**10**) (Bond and Hooper 1969). Authors also claimed that refluxing **9** with 2-bromopyridine in pyridine produced tryptanthrin as the only identifiable product. However, the reaction mechanism for the conversion is not easy to deduce as the authors have agreed.



Although a detailed preparative methods for *N*-(2′-aminobenzoyl)isatins has not been described in the literature, a couple of the cyclization of reaction to tryptanthrins via *N*-(2′-aminobenzoyl)isatins have been reported. The method of Eguchi et al. employed an aza-Wittig reaction of *N*-(2′-azidobenzoyl)isatin and tri(*n*-butyl)phosphine to prepare tryptanthrin (Eguchi et al. 1992), in which *N*-(2′-aminobenzoyl)isatin could be an intermediate.



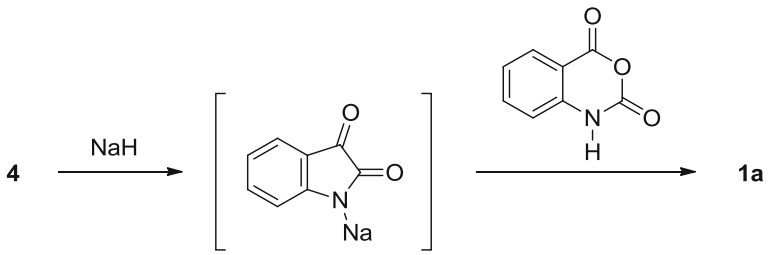
[4] Where the reactions of nucleophilic nitrene complexes to the carbonyl group of aldehydes and ketones to provide the corresponding amines are well described.



The synthetic procedure of Mitscher et al. employed deprotonation of isatins by NaH to form *N*-sodioisatins, which were then allowed to condense with isatoic anhydrides to lead the corresponding tryptanthrins (Mitscher et al. 1981). This procedure has advantages, as many of the starting isatins and isatoic anhydride are not only commercially available but also can be readily prepared by well established procedures (Sandmeyer 1919; Gassman et al. 1977), thus applied to synthesize the derivatives of tryptanthrin (Baiocchi et al. 1993; Grandolini et al. 1997; Valiante 2004; Lee et al. 2007; Gilman et al. 2008).

electrolysis of isatin using Hg cathode at the first reduction potential (−0.9 V vs. CSE) provided tryptanthrin in 92 % yield. The low-energy consumption process may involve a single electron transfer to the oxygen in air, from which a possible reaction mechanism has been deduced as shown in Scheme 2.

Dimerization can also be induced by halogenating agents such as PCl_5 and $POCl_3$ (Moskovkina 1997; Moskovkina et al. 2012; Jao et al. 2008), in which authors claimed 'Baeyer's isatin chloride' (2-chloro-3*H*-indol-3-one, **11**) as a possible intermediate.

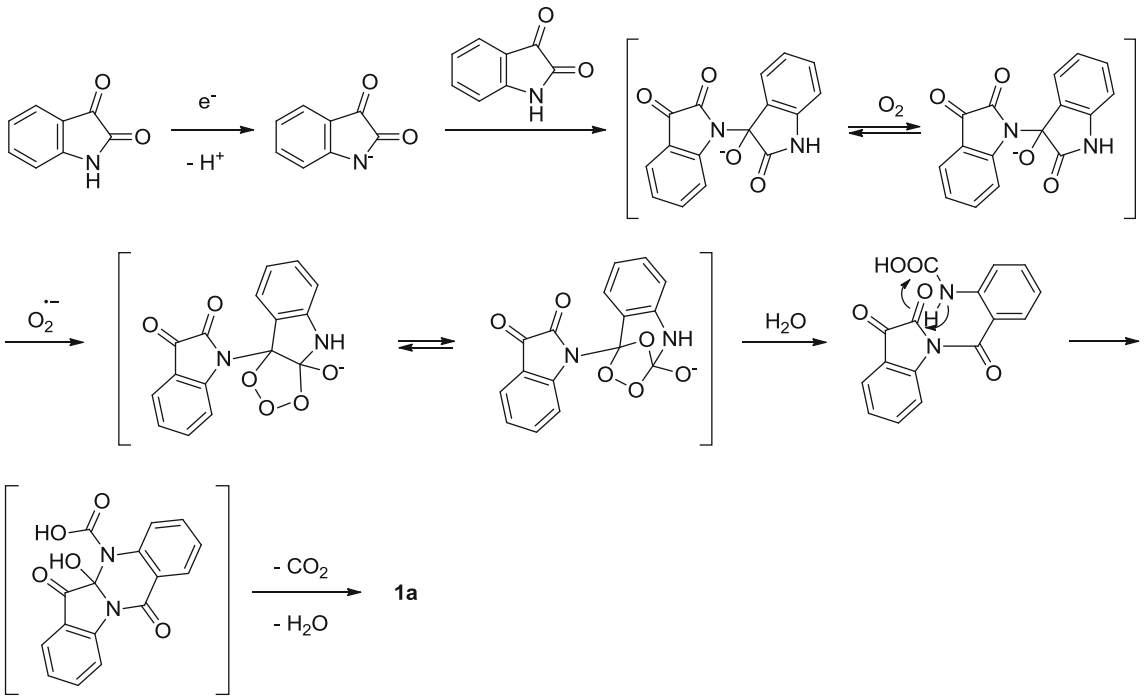


The reaction conditions of this procedure have been modified numerously to improve or simplify such transformation. The experimentally simplest one involves heating isatin (**4**) and isatoic anhydride (**5**) in the presence of an equimolar amount of triethylamine or pyridine (Bergman et al. 1985). Alternative reaction conditions employed involve an inorganic base such as NaOH (Liu et al. 2008) and basic salts such as K_2CO_3 in the presence of microwave (Azizian et al. 2007). In general, excess **5** has been found effective presumably due to the scavenging any water formed during the reaction. Water scavenging agent such as dicyclohexylcarbodiimide (DCC), diiospropylcarbodiimide (DIC), or 1,1′-carbonyldiimidazole has also been employed (Mason et al. 2009). Application of such a procedure to isatin-d_4 and isatoic anhydride-d_4 yielded tryptanthrin-d_8 (Overthür et al. 2002).

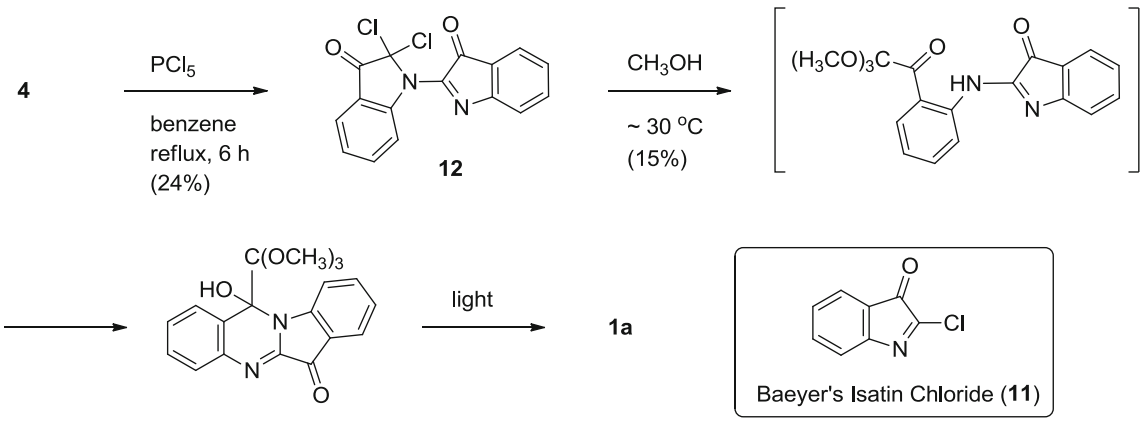
A couple of additional dimerization procedures involves the irradiation of isatin with 10.6 μm wavelength laser light to give tryptanthrin (yield not given) (Karpf and Junek 1978). Batenero and Barba reported electrodimerization of isatin to tryptanthrin (Batanero and Barba 2006). Preparative

Baeyer claimed that the reaction of isatin with PCl_5 afforded isatin chloride as early as in 1878 (Baeyer 1878, 1879) and a variety of reactions of isatin chloride with nucleophiles such as amines, phenols, and 'active methylene' compounds were pursued (Hill and Henze 1924; Begley and Grimshaw 1975; Katritzky et al. 1989). Although, the reaction of **11** with anthranilic acid afforded tryptanthrin (Baker and Duke 1972), isatin chloride remained as a 'phantom' molecule until Cornforth et al. confirmed the structure in 1996 (Cornforth et al. 1996). Cornforth et al. 1996 repeated Baker and Duke's procedure [dilute (4 %) solution of isatin and PCl_5 in benzene and long period of reaction time (6 h)] to get red crystals and confirmed the structure (**12**) by x-ray crystallography. Reaction mechanism of the conversion was proposed as shown in Scheme 3.

Thionyl chloride has long been a condensing agent for the construction of quinazolin-4(3*H*)-one skeleton from anthranilic acid and lactams (Kametani et al. 1977), and has also been applied to the synthesis of tryptanthrin with a



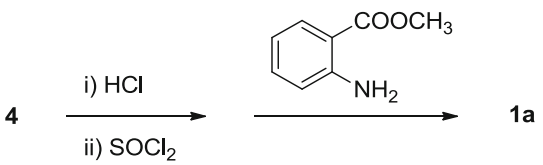
Scheme 2 Possible reaction mechanism for electrodimerization of isatin

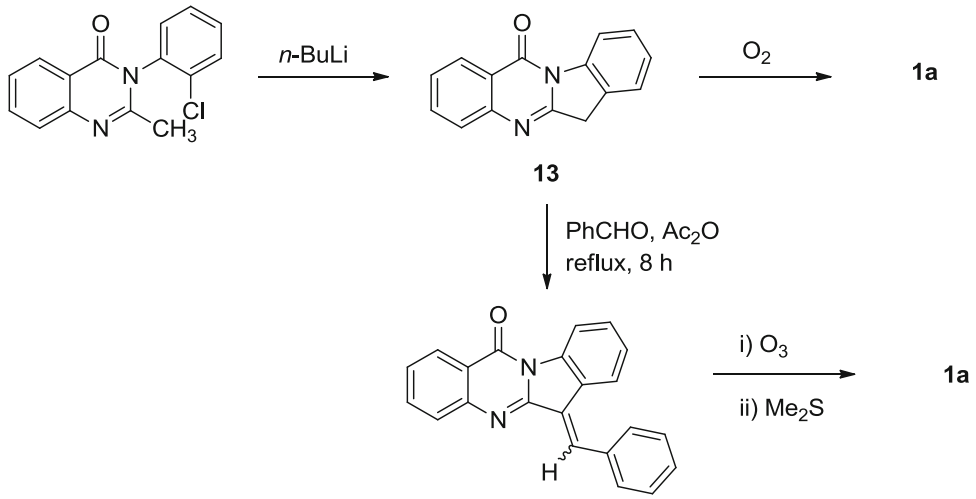


Scheme 3 Dimerization of istain in the presence of PCl_5

modification (Lee et al. 2003). Attempts to develop more efficient and practical synthetic methods for tryptanthrin have been continued and eventually have established a one-pot reaction of isatin and anthranilic acid in the presence of thionyl chloride (Jahng et al. 2008).

Nucleophilic substitution of a carbanion generated from 3-*N*-(2-chlorophenyl)-2-methyl-4(3*H*)-quinazolinone and *n*-BuLi afforded indolo[2,1-*b*]quinaolin-12(6*H*)-one (**13**), to which a keto group at C6 was introduced by either direct air oxidation (Staskun and Wolfe 1992) or Thummel's two-step procedure (Son et al. 2003).

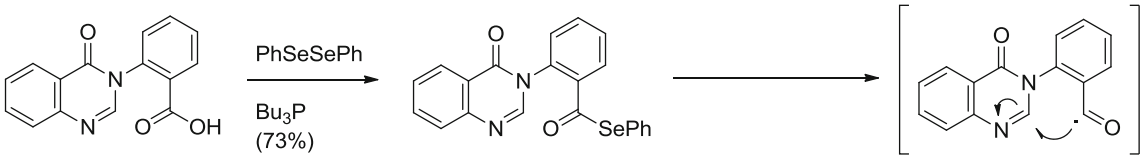




Bowman et al. employed a radical cyclization for the preparation of tryptanthrin (Bowman et al. 2007). The starting material, prepared from 2-(4-oxoquinazolin-3(4*H*)-yl)benzoic acid and 1,2-diphenyldiselane in the presence of tri(*n*-butyl)phosphine, was converted to acyl selenide which was then subjected radical reaction condition to yield tryptanthrin in 3–13 % yield.

deamination of tryptophan would lead 3-(1*H*-indol-3-yl) pyruvic acid, which was then condensed with anthranilic acid to form tryptanthrin (Scheme 4).

Additional studies reveal that *Candida lipolytica* synthesizes tryptanthrin from equimolar amounts of tryptophan and anthranilic acid. Such a procedure allowed to pursue biosynthesis of a series of tryptanthrins by



reaction condition	yield (%)
UV	13
AIBN, UV	11
$(CH_3)_3SnSn(CH_3)_3$, *t*-BuPh, *hv*	3

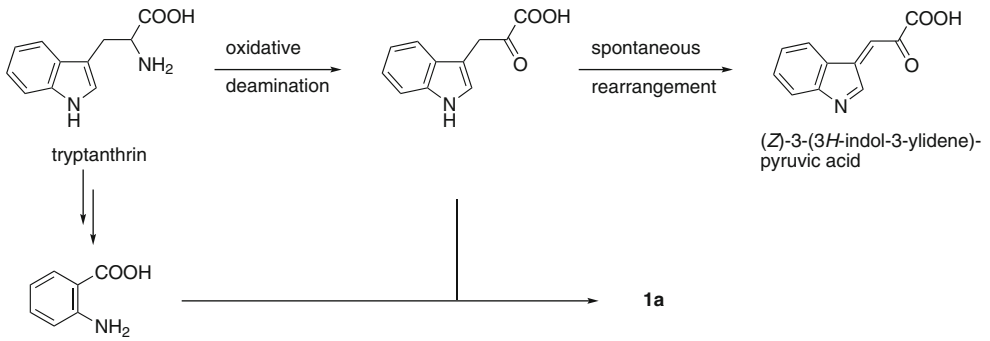
It should be noted that an efficient and green method for the synthesis of tryptanthrin and its derivatives from isatin and isatoic anhydride was recently reported (Kumar et al. 2011), in which the reaction was performed in aqueous medium at room temperature by employing β-cyclodextrin as a catalyst thus opening a new vista in alkaloid synthesis.

Biosynthesis of tryptanthrin

Biosynthetic pathway of tryptanthrin by *Candida lipolytica* has been proposed by Schindler and Zähner as shown in Scheme 4 (Schindler and Zähner 1971). Oxidative

employing either substituted tryptophans and anthranilic acid or tryptophan and substituted anthranilic acids (Fiedler et al. 1976).

Enzymatic hydrolysis of a novel indole *S*,*O*-bisdesmoside, calanthoside, with β-galactosidase in 0.2 M acetate buffer (pH 4.4) provided tryptanthrin in 63 % yield, along with a small amount of indirubin and isatin (Yoshikawa et al. 1998; Murakami et al. 2001). This result may imply that calanthoside is a common genuine glycoside of tryptanthrin and other related alkaloids such as indirubin and isatin in plants. Here again, the reaction mechanism for this conversion was not easy to deduce.



Scheme 4 Proposed mechanism for the possible 1st metabolites of tryptanthrin

Metabolism

Tryptanthrin is an agonist of the rat aryl hydrocarbon receptor (AhR) implying that tryptanthrin can induce cytochrome P450 (CYP) 1A1 as confirmed by immunoblotting and CTP 1A1 activity assay (Schrenk et al. 1997). The LC masses of the two identifiable metabolites from the culture of cytosolic cytochrome P_{450}-mediated metabolism of tryptanthrin were 264 and 280. The first metabolite (M1) was identified as a metabolite monohydroxylated on the aromatic ring of the indole moiety from the MS^2 spectra of protonated tryptanthrin and M1. The structure of M1 was confirmed as 8-hydroxytryptanthrin with a chemically synthesized authentic tryptanthrin (Lee et al. 2007), while the metabolite M2 (mw 280) remained to be characterized (Scheme 4).

The experimental evidence, the reaction mechanism of the cytochrome P_{450}-mediated oxidation process (King 2009), and the electronic aspects of the benzene ring of an indole moiety are enough to deduce a possible metabolic pathway as shown in Scheme 5. Among the possible tryptanthrin epoxides formed by cytochrome P_{450}-mediated oxidation, epoxide **12** is expected to be the most favorable one to undergo either spontaneous rearrangement followed by NIH shift or ring opening by epoxide hydrolase, followed by aromatization to yield 8-hydroxytryptanthirn and 2,8-dihydroxytrypanthrin, respectively, as first metabolites of tryptanthrin. However, it is somewhat surprising that 7-hydroxytryptanthrin (phaitanthrin) (Jao et al. 2008) and 4,8,9-trihydroxytryptanthrin (ophiuroidine) (Utkina and Denisenko 2007) are two hydroxylated tryptanthrins isolated so far, which may imply that an alternative metabolic process operates in nature.

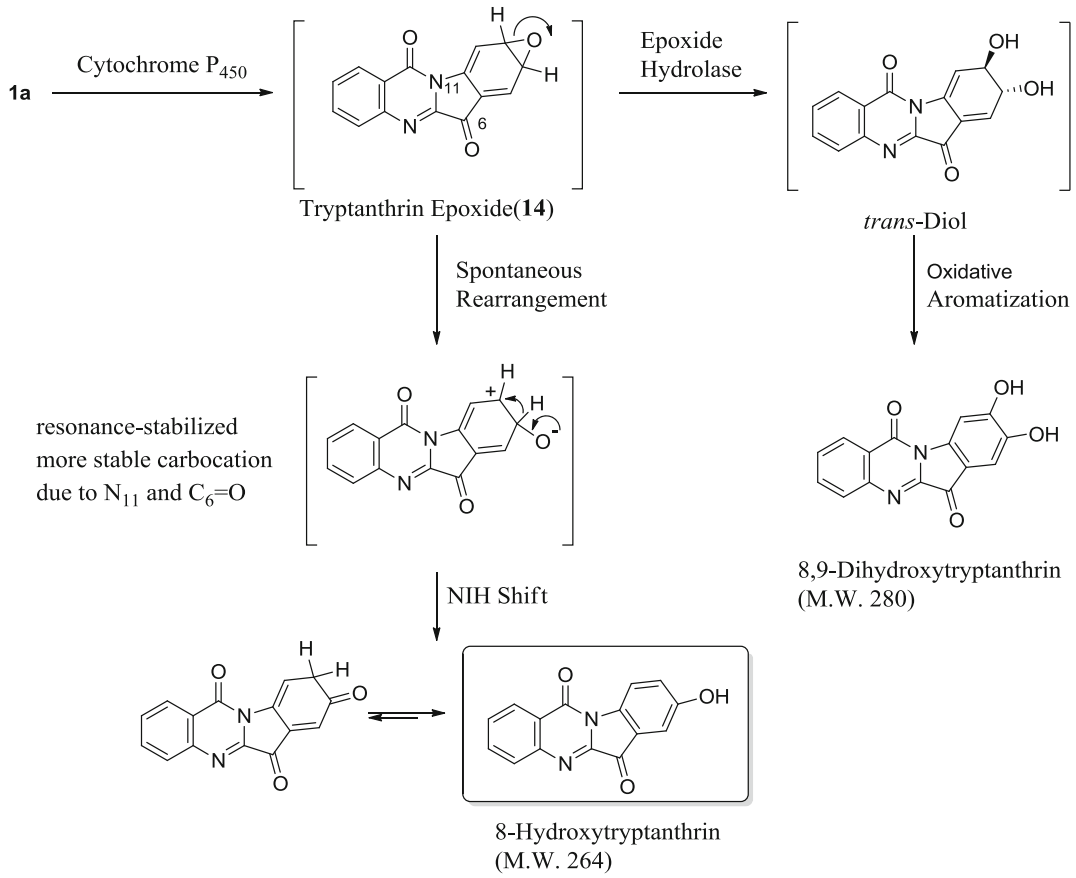
Structural modification and structure–activity relationship

Cytotoxicity

Effect of substituents

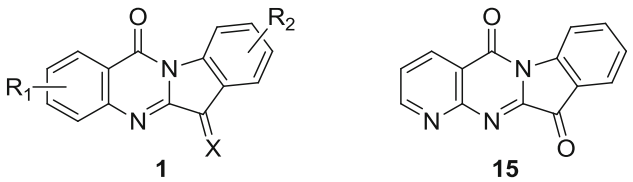
A couple of systematic structural modifications of tryptanthrin have led some promising results for anticancer agents. A substituent at C8 and oxime ethers of C6 carbonyl improved the activity significantly (Table 3).

Promising in vitro activity of the compound **1f** and **1g** allowed testing their in vivo activities. Based on the promising results (optimum T/C 23 %, 17 days) of hollow fibre assay (HFA), **1f** was tested in nude mice bearing HT-29 colon cancer xenograpfts to be active enough to pursue additional studies.



Scheme 5 Proposed mechanism for the possible 1st metabolites of tryptanthrin

Table 3 In vitro cytotoxic activities of indolo[2,1-*b*]quinazolinines (Sharma et al. 2002; Yu et al. 2010)



Compound	R_1	R_2	X	GI_{50}(μM)					
				MCF7/ADR	U251	SW620	SKOV3	DU145	A498
1a	H	H	O	30.0	100	5.0	2.5	0.4	0.95
1b	H	8-Br	O	0.7	>100	4.0	5.0	0.15	3.0
1c	H	8-NH_2	O	5.0	0.7	4.8	5.0	2.0	3.0
1d	H	8-NHAc	O	0.4	20.0	30.0	20.0	0.5	0.4
1e	H	8-$NHCOCH_2Cl$	O	0.06	0.2	0.6	0.05	0.25	0.4
1f	H	H	R[a)]	0.4	0.8	0.75	2.0	2.5	0.6
1g	H	8-NO_2	R[b)]	4.0	2.5	0.5	0.6	2.0	0.09
1h	H	8-Br	R[a)]	0.06	0.45	3.0	3.0	0.2	1.5
1i	H	8-Br	R[c)]	0.3	0.095	3.0	1.0	0.55	2.0
1j	H	8-I	O	48 (95)[d)]	–	–	47 (86)[d)]	–	68 (84)[d)]

Table 3 continued

Compound	R_1	R_2	X	GI_{50}(μM)					
				MCF7/ADR	U251	SW620	SKOV3	DU145	A498
15				77 (95)[d)]	–	–	50 (86)[d)]	–	78 (84)[d)]

[a] N-$OCH_2CH_2N(CH_3)_2$

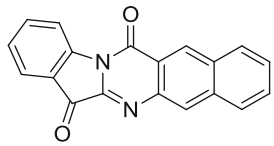
[b] N-$OCH_2CH_2CH_2N(CH_3)_2$

[c] N-$OCH_2CH_2N(CH_2)_5$

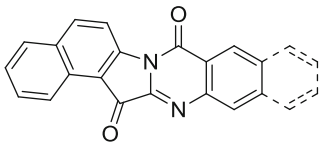
[d] Values are percentage of inhibition

Effect of benzo-annulation

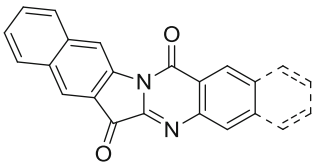
Introduction of additional benzene ring, especially by benzo-annulation would lead the system more delocalized electron state which resulted in changes in chemical and biological properties (Costes et al. 2000; Hong et al. 2010). Benzo-annulated tryptanthrins (**16**) were recently prepared by employing one-pot synthesis from corresponding either (benzo)isatins and (benzo)anthranilic acids in the presence of $SOCl_2$ or (benzo)isatins and (benzo)isatoic anhydrides, in which the prerequisite benzoisatins and benzoisatoic anhydrides could be prepared by employing previously reported methods (Liang et al. 2012).



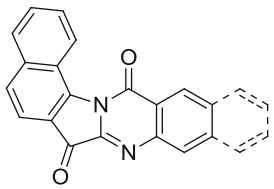
16a



16b,c

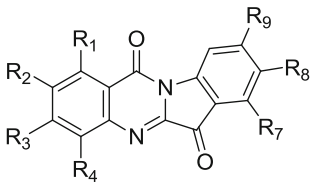


16d,e



16f,g

Table 4 Inhibitory activities of tryptanthrin derivatives on COX-1, COX-2, and NF-κB



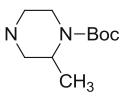
Compound	R_1	R_2	R_3	R_4	R_8	R_9	IC_{50} (μM/L)		
							COX-1	COX-2	NF-κB
1a	H	H	H	H	H	H	2	0.04	1
1k	H	Cl	H	H	H	H	10	2	50
1l	H	H	H	H	Cl	H	1	0.5	5
1m	H	CH_3	H	CH_3	H	H	> 50	> 50	> 100
1n	H	NH_2	H	H	H	H	5	0.5	5
1o	H	H	H	H	COOEt	H	> 50	> 50	> 100
1p	H	H	H	H	F		> 50	> 50	> 100

Table 4 continued

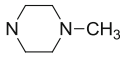
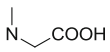
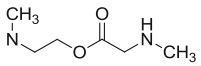
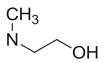
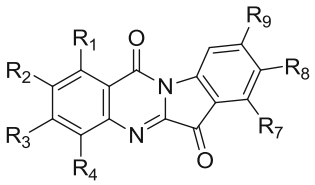
Compound	R_1	R_2	R_3	R_4	R_8	R_9	IC_{50} (μM/L)		
							COX-1	COX-2	NF-κB
1q	H	H	H	OCH_3	SO_2*n*-Octyl	H	>50	>50	>100
1r	H	H		H	F	H	1	1	10
1s	H	H		H	Cl	H	5	0.01	0.5
1t	H	H		H	Cl	H	5	0.5	1
1u	H	H		H	Cl	H	10	0.02	5
1v	H	H		H	OCF_3	H	2	0.5	5

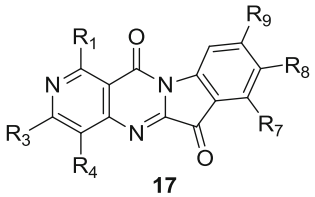
Table 5 Inhibitory activities of tryptanthrin derivatives on *M. tuberculosis* H37Rv



Compound	R_1	R_2	R_3	R_4	R_7	R_8	R_9	MIC (mg/L)
1a	H	H	H	H	H	H	H	1.0
1ab	F	H	H	H	H	H	H	0.25
1ac	H	F	H	H	H	H	H	0.13
1ad	H	H	F	H	H	H	H	0.06
1ae	H	H	H	F	H	H	H	0.13
1af	H	Cl	H	H	H	H	H	0.06
1ag	H	NH_2	H	H	H	H	H	5.0
1ah	H	H	H	H	H	H	OCH_3	0.06
1ai	H	H	H	H	Cl	H	H	0.5
1aj	H	H	H	H	H	Cl	H	0.06
1ak	H	H	H	H	H	H	Cl	0.13
1al	H	H	H	H	H	F	H	0.13
1am	H	H	H	H	H	NO_2	H	0.015
1an	H	H	H	H	H	OCF_3	H	0.03

Although the benzo-annulation on quinazolin-4(3*H*)-one ring did not affect significantly on the inhibitory activities against topo I and II, the benzoannulation on indolin-3-one ring affected the inhibitory activity very much especially by linear annulation. However, bezno-annulated tryptanthrins did not show any significant increase in cytotoxicity against selected human cancer cell lines, which were not directly related either to the inhibitory activities against topo I and II or to the reduction potentials.

Table 6 In vitro antimycobacterial activity of selected C-8 substituted 2-azatryptanthrins against *M. tuberculosis* H37Rv and *M. avium* (MAC) 19075



17

Compound	R_1	R_3	R_4	R_7	R_8	R_9	MIC (mg/L)	
							M. tuberculosis	MAC
17a	H	H	H	H	H	H	0.25	5
17b	H	H	H	H	F	H	0.50	5
17c	H	H	H	H	Cl	H	0.03	0.25
17d	H	H	H	H	CF_3	H	0.015	0.50
17e	H	H	H	H	CH_3	H	0.13	1
17f	H	H	H	H	Et	H	0.03	1
17g	H	H	H	H	*n*-C_4H_9	H	0.015	0.06
17h	H	H	H	H	*n*-C_6H_{13}	H	0.015	<0.2
17i	H	H	H	H	*n*-C_8H_{17}	H	0.015	0.06
17j	H	H	H	H	CHO	H	0.008	0.50
17k	H	H	H	H	2-Octyl	H		
17l	H	H	H	H	cyclohexyl	H		

Table 7 Inhibitory activity of **1a** and its benzo-annulated derivatives (**16**)

Compound	Inhibitory activity (%)				Compound	Inhibitory activity (%)			
	Topo I		Topo II			Topo I		Topo II	
	100 μM	20 μM	100 μM	20 μM		100 μM	20 μM	100 μM	20 μM
1a	68.44	4.09	4.54	N/T	**16e**	41.85	3.60	12.60	N/T
16a	18.79	N/T	15.96	N/T	**16f**	53.41	4.10	14.04	N/T
16b	47.79	4.90	8.13	N/T	**16 g**	84.13	26.30	53.45	19.05
16c	39.10	3.80	71.26	5.83	CPT	63.10	26.30	–	–
16d	69.56	5.40	75.93	5.95	Etoposide	–	–	78.91	36.08

CPT camptothecin

Antiinflammatory activity

Hamburger and Danz prepared 110 derivatives of tryptanthrin and examined their inhibitory activities on COX-1, COX-2 and NF-κB and the results are summarized in Table 4 (Hamburger and Danz 2000; Danz et al. 2001). Introduction of a substituent at C3 (R3) and C8 (R8) as in **1s** and **1u** generally increased inhibitory activities on COX-2 and selectivity on COX-2 while substituents at C4 (R4) (**1o** and **1q**) and C9 (R9) (**1p**) decreased the activity significantly.

Antimalarial activity

One of the early biological properties of tryptanthrin was strong inhibitory activity against microbes for tuberculosis such as *Mycobacterium smegmatis*, *M. tuberculosis*, and *M. avium* (MAC). Mitscher and his coworkers (Baker and Mitscher 1995; Mitscher and Baker 1998a, b) prepared a series of deriviatives of tryptanthrins as well as its aza-analogues to lead a general SAR for antimalarial activity (Tables 5, 6).

Inhibitory activity on topoisomerases

The inhibitory activity of **1a** and its benzoannulated derivatives (**16**) on topoisomerases I and II (topo I and II) were evaluated to show that tryptanthrin and its benzoannulated derivatives showed selective inhibitory activity on topo I with an increase of activity on topo II by benzo-annulation on quinazolin-4(3*H*)-one moiety. Although the benzo-annulation on quinazolin-4(3*H*)-one ring did not affect significantly on the inhibitory activities against topo I and II, the benzoannulation on indolin-3-one ring affected the inhibitory activity very much especially by linear annulations (Liang et al. 2012) (Table 7).

Conclusions and outlook

Despite a long period of time elapsed since its discovery, tryptanthrin continues to be one of the intriguing alkaloids for which more chemistry and biological studies are required. Studies on tryptanthrin are still ongoing to include not only to searches for the new natural sources of itself as well as derivatives of tryptanthrin, developments of more efficient and practical synthetic methods, and structural modifications for stronger biological activities, but also possible candidates for optical usages as photoreceptor.

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