REVIEW

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Pharmacological activities and structure-modification of resveratrol analogues

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Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a well-known natural polyphenol compound. It is reported that resveratrol possesses strong anti-oxidative, anti-inflammatory, cardiovascular protective and cancer chemo-preventive effects. Therefore, there has been a considerable interest in its biological activity, pharmacological activity and also synthetic resveratrol analogues in recent years. Up to now, many new resveratrol derivatives have been synthesized and some new biological activities of these compounds have been found, so in the treatment of Alzheimer's disease and the inhibition of influenza H1N1 neuraminidase. Structure-activity studies revealed that crucial elements of parental components are required for specific effects. This review summarizes the available literatures on the structure-activity relationships and pharmacological properties of resveratrol analogues.

1. Introduction

Resveratrol (*trans*-3, 5, 4′-trihydroxy-trans-stilbene, Fig. 1) is a non-flavonoid polyphenol compound with stilbene structure. It is widely distributed in food and beverages, such as mulberries, peanuts, grapes, and red wine (Jang et al. 1997). Resveratrol, having a wide range of biological activities, can be classified either as a polyphenol or a stilbene. It is produced by plants to protect themselves from damage or infections by heat, ultraviolet, radiation insects, bacteria, and fungi (Bums et al. 2002). In recent years, resveratrol and its derivatives have been reported to exert a variety of biological activities, including chemo-preventive, anti-inflammatory, antioxidant, anti-proliferative, proapoptotic, cardioprotective, anticancer properties, anti-Alzheimer properties (Gusman et al. 2001; Pervaiz and Holme et al. 2009; Villaflores et al. 2012). Accordingly, resveratrol can be developed as chemotherapeutic agent. It is important to note that resveratrol possesses anti-tumor effects. These are mediated through several biological receptors including cyclooxygenase (COX), lipooxygenase (LOX), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), quinone reductase 1 (QR1), quinone reductase 2 (QR2), ornithine decarboxylase (ODC), aromatase and human recombinant cytochrome P450 (Calamini et al. 2008; Boutin et al. 2005; Mikstacka et al. 2012).

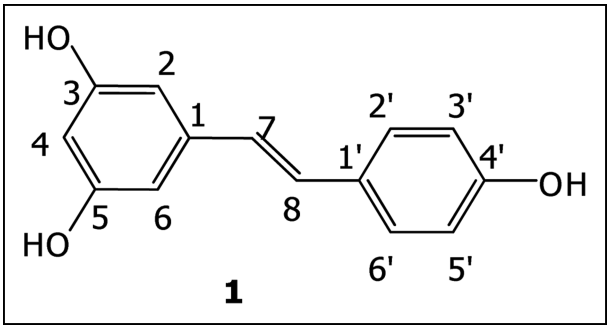

Fig. 1: The structure of resveratrol.

Through their pharmacophores, resveratrol and its derivatives could act on multiple biological receptors. At present, there are mainly five methods for synthesizing resveratrol and its derivatives, namely Perkin reaction, Wittig reaction, Wittig-Horne reaction, Heck-coupling and McMurry reaction (Dhyani et al. 2011; Gester et al. 2005; Hansen et al. 2010; Murphy et al. 1998; Solladie et al. 2003). We can use different synthetic methods to obtain novel derivatives with different activities through structural modification of resveratrol. Structure-activity studies would provide a basis for the development of novel resveratrol analogs with more potent antitumor and antioxidant activities or other properties through selective modification of the stilbene scaffold of resveratrol.

2. Modification of benzene ring

In early studies, many researchers suggested that the 4-hydroxy in the *trans*-conformation on 4- and 4′-positions of the stilbenic backbone is crucial for the anti-proliferative effect of resveratrol (Stivala et al. 2001). Analysis of structure-activity relationships had also revealed that the replacement of hydroxyl of resveratrol with methoxy substantially potentiated resveratrol's cytotoxic activity (Lee et al. 2003). Therefore, a series of methoxylated

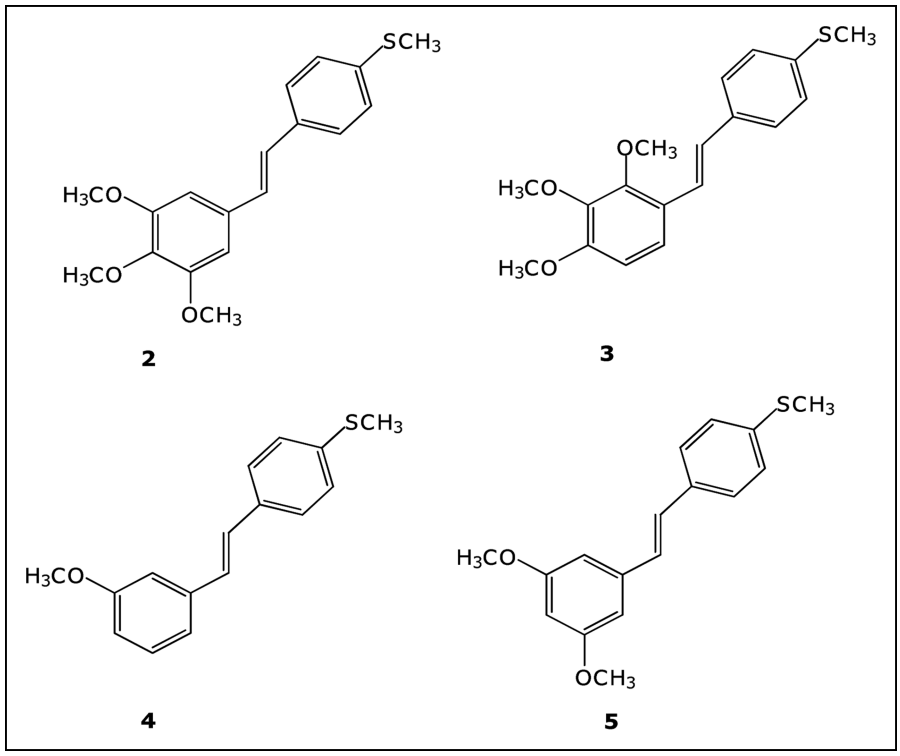

Fig. 2: The structure of 4′-methylthio resveratrol derivatives.

analogs of resveratrol were prepared to increase the antitumor activity of resveratrol.

2.1. 4′-Methylthio resveratrol derivatives

Mikstacka et al. (2012) synthesized several 4′-methylthio-analogues (Fig. 2) and reported that 4′-methylthio-derivatives were effective inhibitors of all P450 enzymes, among which 3, 4, 5-tri- methoxy-4′-methylthio-*trans*-stilbene (compound **2**) and 2, 3, 4-trimethoxy-4′-methylthio- *trans*- stilbene (compound **3**) showed great P450 enzymes-inhibition (Mikstacka et al. 2012). It also suggested that the most probable binding poses of the methylthiostilbene derivatives in CYP1A2 active sites were those with the methylthio substituent directed against the heme iron. Therefore, the 4′-methylthio substituent is crucial for resveratrol analogues as inhibitors on P450 s.

Early studies have indicated that the key role in inflammation is functioned by transcription factors, such as NF-κB and the activator protein (AP-1), both of which control the expression of inflammatory mediators, including cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Poser et al. 2004). It has been shown that resveratrol exerts its anti-inflammatory activity through inhibiting NF-κB and AP-1 (Chung et al. 2011). Szaefer et al. (2014) synthesized two 4′-methylthio-derivatives (compounds **4** and **5**) showing that methylthiostilbenes might be equal or more potent modulators of NF-κB, AP-1 and other transcription factors than resveratrol (Szaefer et al. 2014). However, compound **4** is a more efficient inhibitor of NF-κB, and compound **5** has a higher cytotoxicity of these thiomethylstilbenes. Moreover, it was also shown that the substitution of 4′-methoxy group in the stilbene molecule with a less electronegative sulfur atom reduces toxicity for HEK 293 cells, enhancing the compound's ability to activate human SIRT1 (is considered to be responsible for cell longevity) (Yang et al. 2007; Park et al. 2013). Those results indicated that different numbers and positions of the methoxy group would influence cytotoxicity of the compounds.

2.2. Amination of resveratrol derivatives

In many research works, amino groups were introduced into resveratrol analogues (Fig. 3) and their biological activities were evaluated. Sun et al. synthesized a series of amine derivatives and found that compound **6** with a *para*-amino group has displayed versatile biological activities including nitric oxide synthase inhibition, aromatase inhibition ($IC_{50} = 22\ \mu M$), and inhibition of TNF-a-induced NF-κB activity (Sun et al. 2010). Both aromatase and QR2 have been targeted for discovery and identification of chemopreventive agents (Calamini et al. 2008). From preliminary structure-activity relationship studies and molecular modeling results, it seems that the *para*-amino group on the *trans*-stilbene benzene ring is essential for aromatase inhibitory activity, and the introduction of an imidazole moiety improves activity greatly. Compounds **7** and **8** also displayed potent QR2 inhibitory activity, and their complex crystal structures with QR2 have been determined. Compound **9** was the most potent aromatase inhibitor of the amine derivatives, with an IC_{50} value of 36 nM (Sun et al. 2010). Sun et al. (2010) indicated that the position of amino group on ring A was of greater importance than that of the methoxy groups (ring B), and a *para*-amino group was better than an *ortho*- or *meta*-amino group.

In recent years, many studies have found that resveratrol analogues had some functions as anti-AD agents. Lu et al. (2013) synthesized some resveratrol derivatives and evaluated their biological activities. Their study results showed that compounds **10** and **11** significantly inhibited self-induced β-amyloid (Aβ) aggregation and Cu (II)-induced Aβ1-42 aggregation. Furthermore, compound **11** crossed the blood-brain barrier (BBB) *in vitro* and did not exhibit any acute toxicity at a high dose in mice (Lu et al. 2013).

2.3. Hybrid derivatives of resveratrol with other compounds

Aldawsari et al. (2014) combined resveratrol with salicylate, yielding a series of new hybrid derivatives and those

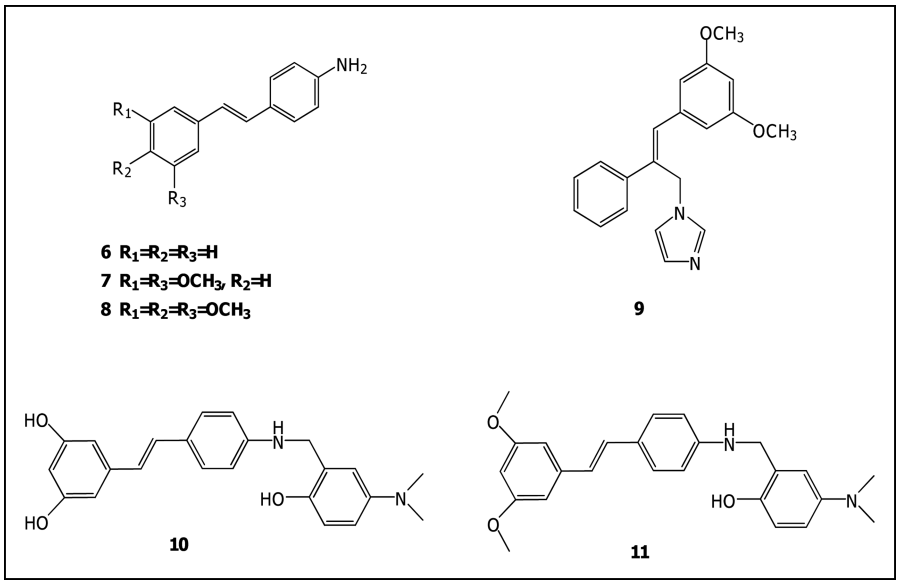

Fig. 3: Structure of amination resveratrol derivatives.

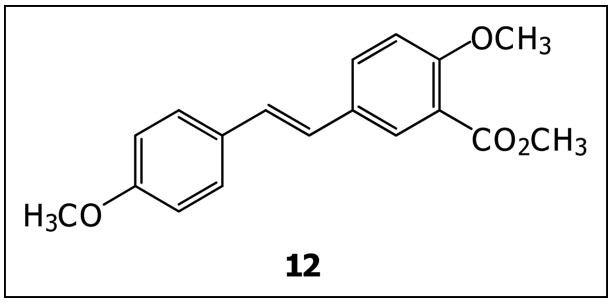

Fig. 4: Structure of compound **12**.

compounds were evaluated as potent CYP1A1 inhibitors, particularly compound **12** (Fig. 4). Moreover the study indicated that a carboxylate group on ring A and a methyl ester are the key groups of resveratrol derivatives. On the other hand, compounds having a free carboxylic acid group could increase the catalytic activity of the enzyme.

2.4. Digalloyl resveratrol

Bernhaus et al. (2009) synthesized a new phenolic acid derivative (compound **13**, Fig. 5) by introducing digalloyl group into resveratrol (Bernhaus et al. 2009). This study indicated that compound **13** could inhibit HT-29 human colon cancer cells.

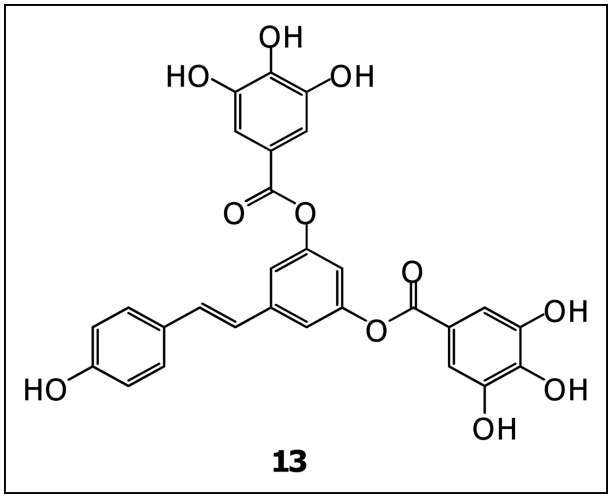

Fig. 5: Structure of digalloyl resveratrol.

2.5. Resveratrol oligomers

There are various naturally occurring stilbene-like compounds which are structurally related to resveratrol. For example, the resveratrol oligomers (Fig. 6), which are characterized by the polymerization of two to eight or even more resveratrol units, are the largest group of oligomeric stilbenes. They showed multiple biological activities, some of which in activity, stability

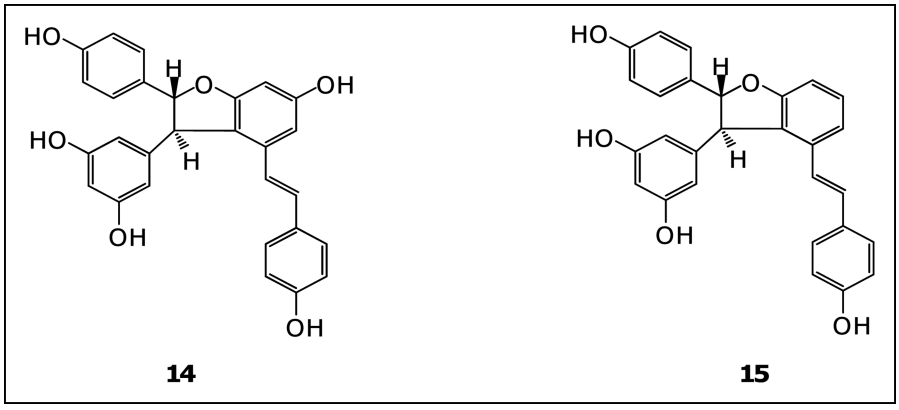

Fig. 6: Structure of resveratrol oligomers.

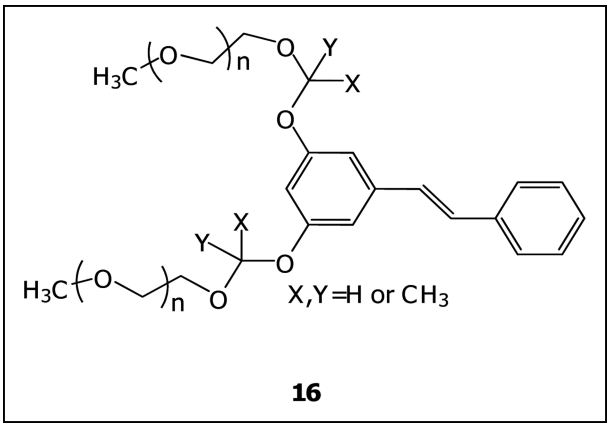

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Fig. 7: Structure of acetal-resveratrol derivatives.

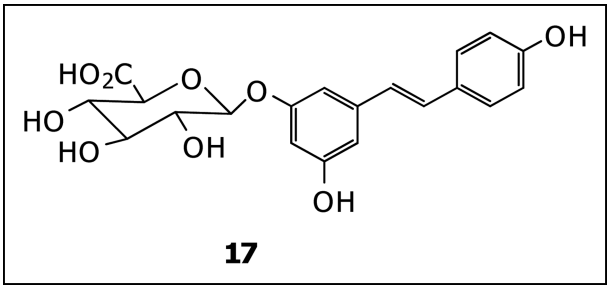

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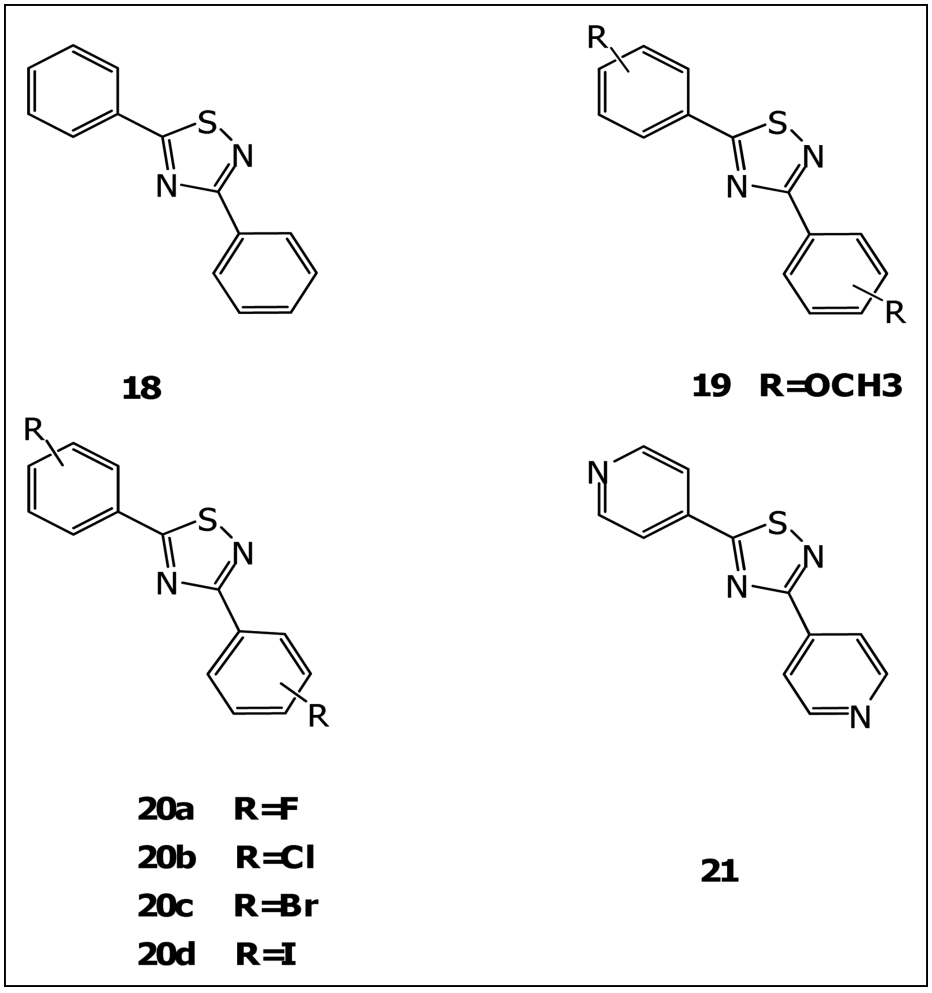
Fig. 8: Structure of resveratrol 3-O-β-D-glucuronide.

and selectivity are superior to resveratrol (Xue et al. 2014). It was reported that compound **14** possessed better antioxidant properties to O_2-radicals (IC_{50} value of 0.12 to 0.16 mM) than resveratrol (IC_{50} value of 0.92 to 0.98 mM) and could inhibit reactive oxygen species production (Kim et al. 2002; Privat et al. 2002). In addition, compound **14** showed a better anticancer activity, existing anti-proliferative effect as well as pro-apoptotic effects on many tumor cells, such as leukemic cells, Hep G2 cell, and human colon cancer cell lines (Barjot et al. 2007; Colin et al. 2008; Colin et al. 2009). However, compound **15** which is an isomer of compound **14,** only exist in plants in quite a low amount.

2.6. Acetal derivatives of resveratrol

Although resveratrol possesses a variety of positive health effects, the pharmacological exploitation of resveratrol is hindered by rapid phase-II conjugative metabolism in enterocytes and hepatocytes. Mattarei et al. (2013) reported the synthesis and characterization as well as the assessment of *in vivo* absorption and metabolism of a set of pro-drugs of resveratrol, in which compounds the OH groups are engaged in linking to the formal ($-OCH_2OR$) or the more labile acetal ($-OCH(CH_3)OR$) (Mattarei et al. 2013). They also indicated that compound **16** (Fig. 7) having maximal absorption may be a convenient tool for systemic delivery of the unconjugated parent compound.



18

19 R=OCH3

20a R=F
20b R=Cl
20c R=Br
20d R=I

21

Fig. 9: The structure of heterocycle derivatives of resveratrol.

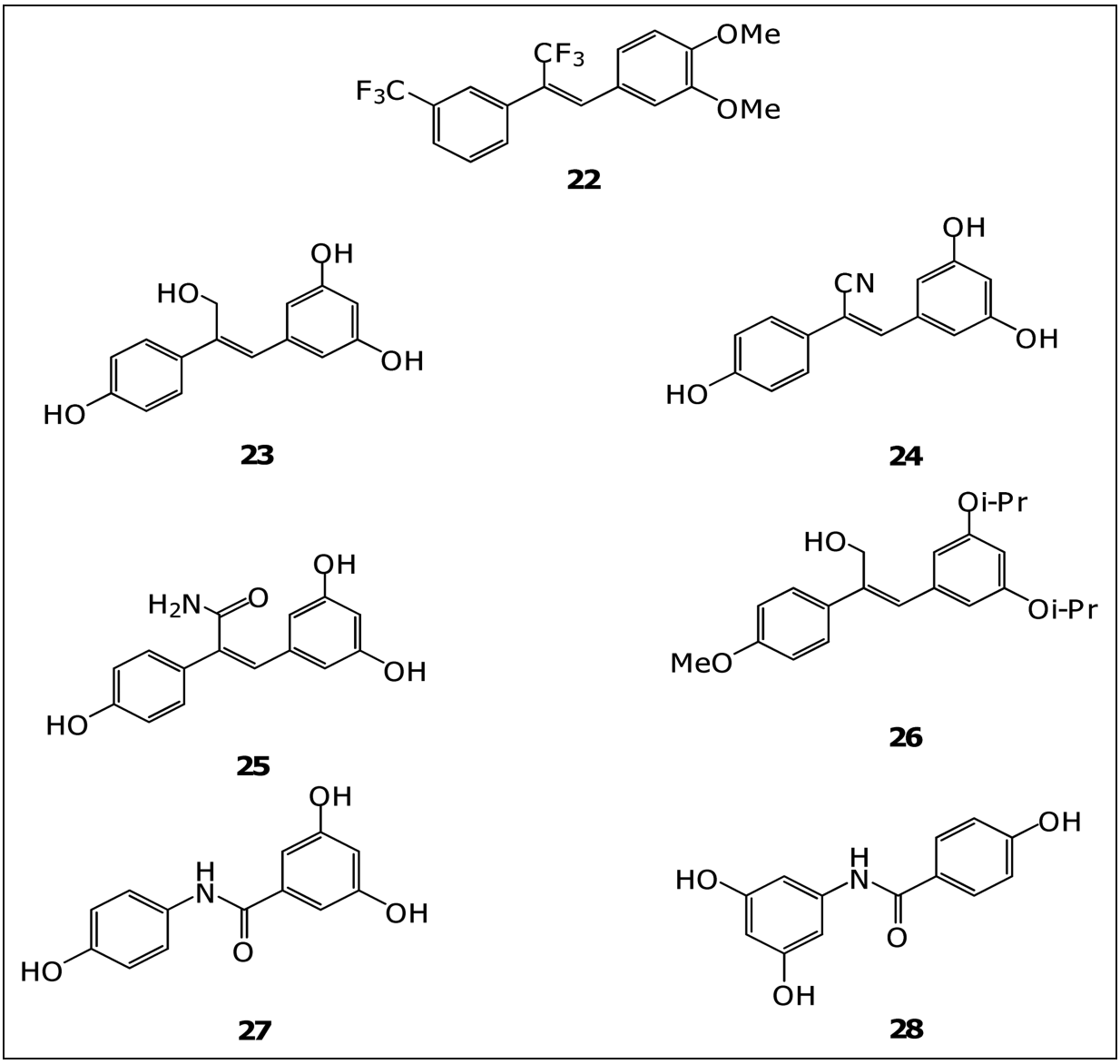


Fig. 10: The structure of olefin-substituted resveratrol derivatives.

2.7. Glycosylated resveratrol derivatives

Resveratrol is an important component of the antioxidant and defense system in plants. The absorption of resveratrol and its derivatives is facilitated by their conjugation with carbohydrates, which causes an increase in the hydrophilicity of those compounds (Mathew et al. 2012). Therefore, the effect of glycosylation of resveratrol has also been examined (Regev-Shoshani et al. 2003). It was revealed that, glycosylation at the p-hydroxy or m-hydroxy group by glucose confers protection from enzymatic oxidation and extends its half-life in the cell (Regev-Shoshani et al. 2003). Recent evidence indicates that compound **17** (resveratrol 3-*O*-β-D-glucuronide, Fig. 8), a resveratrol analogue conjugated with glucose, shows the highest concentration in natural stilbenes and possesses, anti-cholesteric, antioxidant and cardio-protective properties (Mathew et al. 2012; Ryu et al. 2002).

3. Modification of double bond

3.1. Heterocyclic derivatives of resveratrol

Mayhoub et al. (2012) synthesized a series of thiadiazole analogues (Fig. 9). Replacement of the stilbene ethylenic bridge of resveratrol with a 1, 2, 4-thiadiazole heterocycle (compound **18**) showed an increased inhibition of aromatase, NF-κB and QR1. Modification with two methoxy groups or halogen atoms on the two aromatic rings or a substitution with pyridine (compounds **19**, **20**, **21** respectively) does enhance potencies and selectivities, particularly compound **21** (the most active). In addition, this result also showed that 4, 4′-disubstituted compounds possessed the best activities compared with substitutions at other positions.

3.2. Olefin-substituted resveratrol derivatives

Early studies indicated that compound **22** (Fig. 10) is the most potent inhibitor of QR2 (IC_{50} value of $0.18 \pm 0.3\,\mu M$) (Kang et al. 2009). St. John et al. (2009) synthesized a series of novel analogues of resveratrol with electron-donating (OH, OMe, and NMe_2 etc.), electron-withdrawing (F, CF_3 and CN etc.) and naphthyl substituents, some of which were identified to potently inhibit QR2 with increased affinity and to serve as leads of cancer chemo-preventive or anticancer drug for the future development of QR2 inhibitors, especially compounds **23**, **24** and **25** (IC_{50} of $5.1 \pm 0.3\,\mu M$, IC_{50} of $5.9 \pm 0.3l\,\mu M$, IC_{50} of $9.3 \pm 1.9\,\mu M$ respectively). They also identified a novel binding orientation

of the E-configured resveratrol analogue compound **26**, which is the first example of an E-configured resveratrol analogue bound in the QR2 active site. In addition, compounds **27** and **28,** the two benzanilide resveratrol analogues were also found to inhibit QR2. However, the IC_{50} data showed that the position of the amide nitrogen relative to the resorcinol ring has a measurable effect on the ability of the benzanilide analogue to inhibit QR2: when the nitrogen is directly bound to the resorcinol ring, the IC_{50} decreased roughly 40% (16.8 ± 1.3l μM *vs* 27.6 ± 4.6l μM). Those results indicated that the electron-withdrawing CN or CF_3 could enhance the ability of olefin-substituted resveratrol derivatives to inhibit QR2.

4. Conclusion

Due to the multiple beneficial effects of resveratrol, during the past several years, research works were focused on the investigation of biochemical effects of resveratrol. In recent years, many novel resveratrol derivatives have been found by chemical synthesis and biosynthesis. Meanwhile, wide biological activities of these compounds have been suggested. Moreover, many of those new derivatives showed increased potency and reduced toxicity or more selective activities compared to the parental resveratrol. Affinity of those compounds for acceptors was influenced by substituent positions, particularly 3, 5-positions and 4'-position. Overall, the research on the structure–activity relationship of resveratrol derivatives is a significant method to yield desired compounds which possess higher effects of anti-tumor, anti-bacterial, oxidation and anti-Alzheimer's disease. Many of the synthesized resveratrol derivatives displayed promising biological activities and might therefore be further investigated.

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