Glutathione *S*-transferase- and acetylcholinesterase-inhibiting natural products from medicinally important plants*

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Abstract: Naturally occurring enzyme inhibitors play an important role in a drug discovery program. Glutathione *S*-transferases (GSTs) play a significant role in the detoxification and metabolism of many xenobiotic and endobiotic compounds. GSTs are considered to be responsible for decreasing the effectiveness of anticancer/antiparasitic agents used for the treatment of cancer and parasitic diseases. The effectiveness of these biomedical agents may be improved by using GST inhibitors as an adjuvant during chemotherapy. Acetylcholinesterase (AChE) inhibitors have potential applications in curing cardiac problems and Alzheimer's disease. This article describes the identification of natural products exhibiting GST and AChE inhibitory activities, from medicinally important plants. Results obtained from the structure–activity relationship (SAR) studies of some of these newly discovered enzyme inhibitors are also discussed.

Keywords: acetylcholinesterase; glutathione *S*-transferase; *Buxus hyrcana*; *Barleria prionitis*; *Caesalpinia bonduc*; *Mucor plumbeu*; *Artocarpus nobilis*.

INTRODUCTION

Plant natural product chemistry has played an active role in generating a significant number of drug candidate compounds in a drug discovery program [1,2]. For instance, taxol, one of the best known anticancer agents, was isolated from a Pacific yew tree, *Taxus brevifolia* [3]. Recently, it has been reported in the literature that approximately 49 % of 877 small molecules that were introduced as new pharmaceuticals between 1981 and 2002 by New Chemicals Entities were either natural products or semi-synthetic analogs or synthetic products based on natural product models [2]. Currently, natural product chemists are actively involved in developing new in vitro bench-top bioassays which allow for the practical applications of bioassay-guided fractionations of crude extracts of plants, marine organisms, and microbes in order to discover new pharmaceuticals. One of these aspects is to discover new naturally occurring enzyme inhibitors. These enzyme inhibitors may either be used as adjuvant to improve chemotherapy or to treat various diseases.

 Presently, a lot of concern has been raised about anticancer drug resistance. Several research investigations have been conducted in the past in a bid to explain the mechanisms of acquired drug re-

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sistance in the treatment of cancer and parasitic diseases. These studies have indicated that cystosolic detoxification enzyme, glutathione *S*-transferases (GSTs; E.C. 2.1.5.18), are playing an active role in this process [4]. GSTs are phase II detoxification isozymes that function in the conjugation of a wide variety of exogenous and endogenous electrophilic substances to glutathione. Glutathione is a tripeptide having γ-glutamyl-cysteinyl-glycine amino acids in its back bone. This glutathione adduct formed is less toxic and has a very high solubility in water. This adduct can easily be excreted from the body [5]. Anticancer drugs have electrophilic centers and can easily form this adduct in the presence of GST and will be excreted from the body. This would lower the efficiency of the chemotherapeutic agent. To increase the effectiveness of cancer chemotherapeutic agents, it might be necessary to use GST inhibitors as adjuvant during chemotherapy. Natural products for GST inhibition have not been explored extensively. It is a worthwhile to discover natural products exhibiting GST inhibitory activity with a view to using them as an adjuvant during cancer chemotherapy.

Acetylcholine is a neurotransmitter at all preganglionic autonomic, parasympathetic, and some sympathetic postganglionic nerve endings, the neuromuscular junction and at some CNS synapses [6]. Acetylcholinesterase (AChE) is an enzyme that degrades acetylcholine and is used as a marker for cholinergic neural function. AChE inhibitors can be used to prevent this degradation of acetylcholine, and this is considered to be one of the most promising symptomatic therapeutic approaches for the treatment of Alzheimer's disease. Based on this hypothesis, AChE inhibitors have extensively been studied over the last two decades, and this has resulted in the discovery and development of several natural and synthetic AChE inhibitors [7].

Based on the importance of these two enzymes, we are involved in discovering bioactive natural products exhibiting GST and AChE inhibitory activities. To achieve this goal, we screened the crude extracts of a number of medicinally important plants including *Barleria prionitis*, *Caesalpinia bonduc*, *Artocarpus nobilis*, and *Buxus hyrcana* for GST and AChE inhibitory activities. It was discovered that the crude ethanolic extracts of *B. prionitis*, *A. nobilis*, and *C. bonduc* have shown GST inhibitory activities with IC_{50} values of 160, 56, and 83 µg/ml, respectively. The crude ethanolic extracts of *B. prionitis*, *A. nobilis*, and *B. hyrcana* were found to be active in AChE inhibition assay with IC_{50} values of 192, 80, and 45 µg/ml, respectively. During these studies, we also found that the ethyl acetate extract of a fungus, *Mucor plumbeus* exhibited AChE inhibitory activity with an IC_{50} value of 86 µg/ml. The detailed chemical studies on these aforementioned crude extracts have resulted in the isolation of a number of natural products exhibiting GST and AChE inhibitory activities. These results are summarized as follows.

PHYTOCHEMICAL STUDIES ON *BARLERIA PRIONITIS*

Barleria prionitis is an annual shrub, 1–3 feet high. This plant is distributed throughout Africa, India, Sri Lanka, and tropical Asia [8]. *B. prionitis* Linn (Acanthaceae) is locally known as "Vajradanti" in India and "Katukaradu" in Sri Lanka. This plant has been used extensively in folk medicines for the treatment of various ailments. For instance, the dried plant is used to treat whooping cough and asthma in infants and children. The leaves are chewed to relieve toothache, and the paste of the root is applied to disperse boils and glandular swellings. In India and Thailand, the decoction of leaves and flowers of *B. prionitis* is used in the treatment of viral fever [9]. The plant showed biological activity against respiratory syncytial virus [10] and has also been reported as anti-arthritic, anti-inflammatory, and anti-fertility agent [11]. The aqueous bioactive fractions are reported to possess hepatoprotective, antistress, and immunorestorative properties [12].

The crude extract of this plant exhibited GST inhibitory activity with an IC_{50} value of 160 µg/mL. Our detailed phytochemical studies on the crude ethanolic extract of *B. prionitis* have resulted in the isolation of six natural products: balarenone (**1**), pipataline (**2**), lupeol (**3**), prioniside A (**4**), prioniside B (**5**), and prioniside C (**6**). Compounds **1**, **4**, **5**, and **6** were new natural products, while **2** and **3** were

known compounds. Compounds **1–6** showed GST inhibitory activity with IC_{50} values of 48.50, 57.0, 60.0, 49.26, 22.91, and 12.44 µM, respectively. Compounds **1–6** were also screened for AChE inhibitory activity and found to be active in this bioassay with IC_{50} values of 200.09, 135.00, 89.97, 175.98, 133.46, and 50.03 µg/ml, respectively. Pipataline (**2**) was isolated on a large scale, and it exhibited moderate bioactivity in both of our enzyme inhibition assays. We attempted to study its structure–activity relationships (SARs) by preparing its different structural analogs (**7–8**). 7,8-Epoxypipataline (**7**) was synthesized by doing an epoxidation reaction on the C-7/C-8 double bond of pipataline (**2**) using *m*-chloroperbenzoic acid. Compound **7** was further reacted with 20 % ammonium hydroxide solution using household microwave radiation as a catalyst to afford 7-amino-8-hydroxypipataline (**8**). Both of these compounds were found to be active in AChE inhibition assay with IC_{50} values of 164.00 and 36.75, respectively. This SAR study suggested that the AChE inhibitory activity of compound **8** was significantly increased as compared to parent compound **2**, and this increase in bioactivity may be due to the presence of an amino functionality at C-7 [13].

(1)

(2)

(3)

(6)

(4) R = OH
(5) R = OAc

(7)

(8)
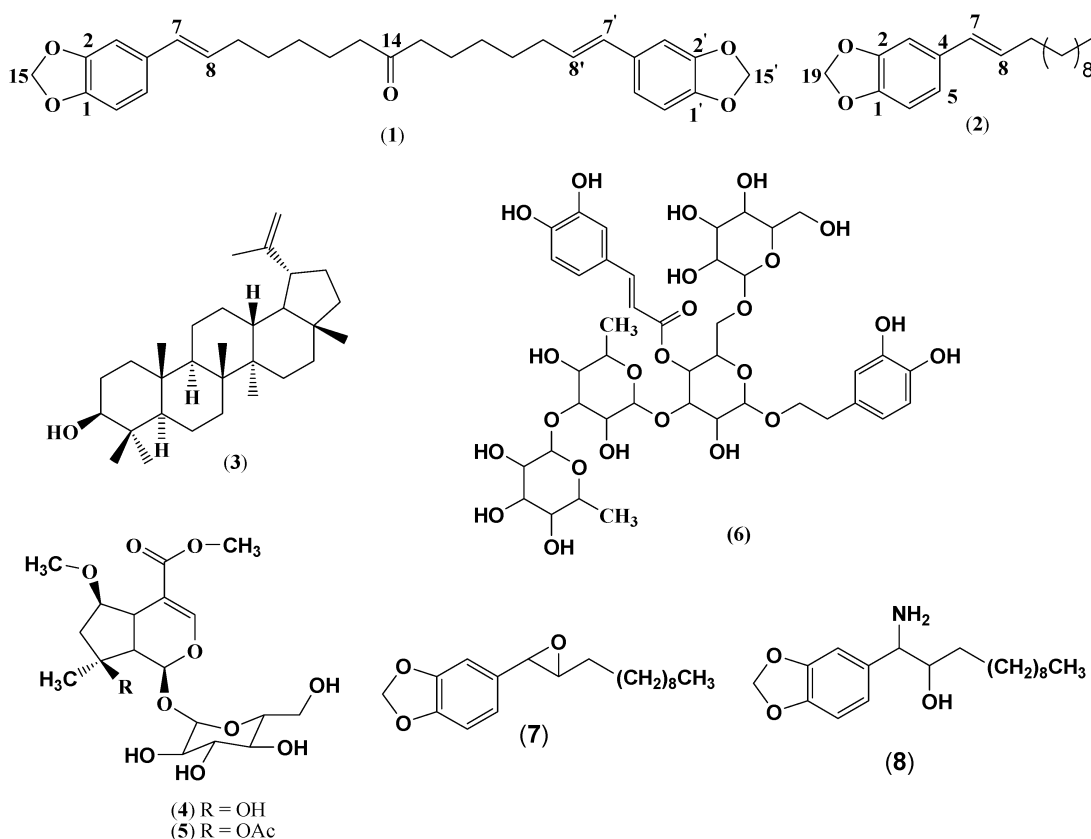
BIOACTIVE CHEMICAL CONSTITUENTS OF *CAESALPINIA BONDUC*

Caesalpinia bonduc L. (Fabaceae) is a medicinal plant and is widely distributed in the tropical and subtropical regions of Asia and the Caribbean. This plant has many applications in indigenous systems of medicine in the aforementioned regions. For instance, its seed and bark extracts have been used as antihelmintic, anticancer, antimalarial, hypoglycemic, anti-inflammatory, antimicrobial, antirheumatic, and antipyretic agents [14,15]. Previous phytochemical analyses of *C. bonduc* and other plants of this family have resulted in the isolation of several cassane and norcassane furanoditerpenes [16]. A few of them

exhibited inhibitory activity against interleukin-1 production and growth inhibitory activity against malaria-causing *Plasmodium falciparum* [17,18]. Our phytochemical studies on the ethanolic extract of this plant afforded a new sterol, 17-hydroxy-campesta-4,6-dien-3-one (**9**) as well as four known compounds, 13,14-*seco*-stigmasta-5,14-dien-3α-ol (**10**), 13,14-*seco*-stigmasta-9(11),14-dien-3α-ol (**11**), caesaldekarin J (**12**), and pipataline (**2**) as GST inhibitors. All of these compounds (**9–12**) showed GST inhibition activity with IC$_{50}$ values of 380, 230, 248, and 250 μM, respectively [19]. The IC$_{50}$ value of sodium taurocholate, a standard steroidal GST inhibitor, under these conditions was found to be 398 μM. This bioactivity data suggested that the bioactivity of compounds **10–12** was significantly higher than that of sodium taurocholate.
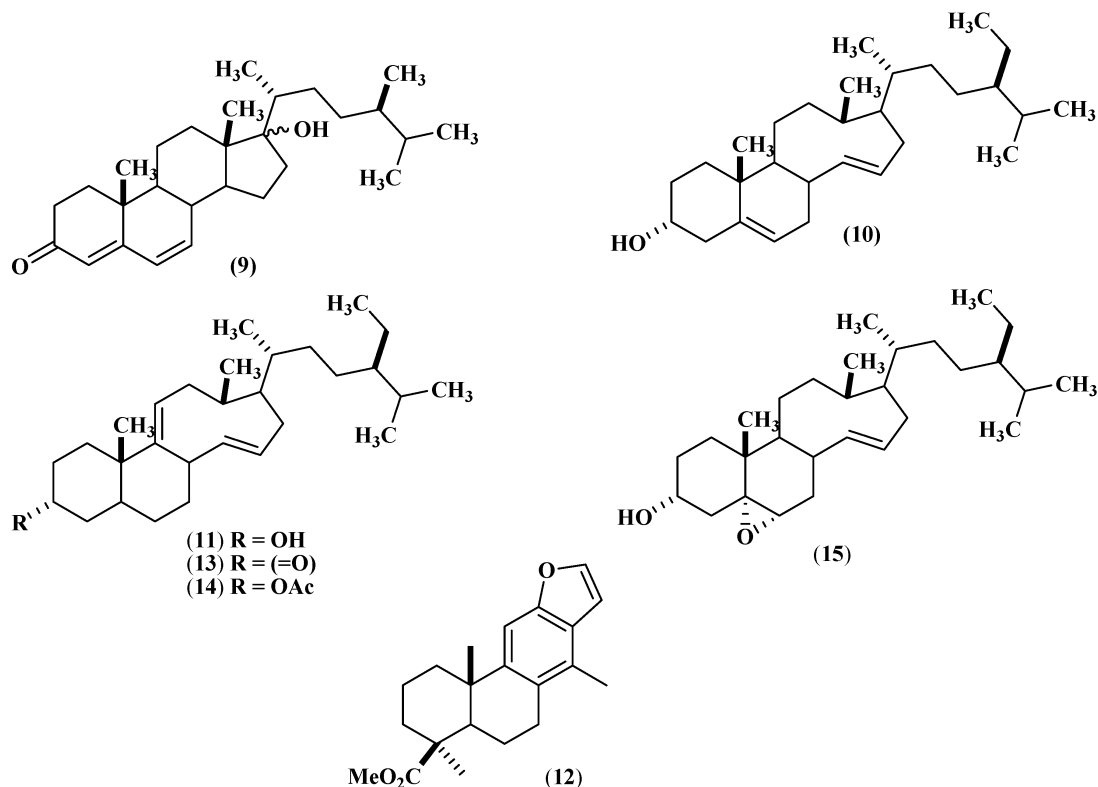

We prepared different derivatives of compounds **10** and **11** in order to study their SARs. Compound **13** was synthesized by doing pyridinium chlorochromate (PCC) oxidation of the C-3 hydroxyl group in **10**, while **14** was prepared by reacting the parent compound **10** with acetic anhydride in pyridine. Compound **15** was prepared by doing an epoxidation reaction on the C-5/C-6 double bond with *m*-chloroperbenzoic acid in compound **11**. Compounds **13–15** were also evaluated for GST inhibitory activity and found to be active in this assay with IC$_{50}$ values of 158, 153, and 118 μM. This bioactivity data suggested to us that a keto or an ester functionality at C-3 in compounds **13** and **14** might be playing a significant role in increasing the GST inhibitory activity. The increase in bioactivity of compound **15** was hypothesized to be caused by the presence of an epoxide functionality at C-5/C-6. These findings further suggested that studies on SARs of bioactive compounds are sometimes worthwhile to have a compound with increased bioactivity.

BIOACTIVE COMPOUNDS FROM *ARTOCARPUS NOBILIS*

Artocarpus nobilis is a tree of moderate size and is the only endemic species of this genus, *Artocarpus*, found in Sri Lanka [20]. This plant is reported to contain cycloartane-type triterpenoids, flavonoids, benzofurans, and stilbene derivatives [20–23]. A few of them have shown antifungal and antioxidant activities. For instance, 2',4,'4-trihydroxy-3'-[6-hydroxy-3,7-dimethyl-2(*E*), 7-octadienyl]chalcone and 2',4',4-trihydroxy-3'-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone were reported to exhibit antifungal activity against *Cladosporium cladosporioides* and radical scavenging activity [23].

The crude ethanolic extract of *A. nobilis* exhibited GST and AChE inhibitory activities with IC_{50} values of 125 and 110 µg/ml, respectively. Our recent phytochemical studies on the crude ethanolic extract of this plant resulted in the isolation of two new cycloartane-type triterpenoids, artocarpuate A (**16**), and artocarpuate B (**17**) along with two known flavonoids, artoblidxanthone (**18**) and artoninse (**19**). Compounds **18** and **19** have shown GST inhibitory activity with IC_{50} values of 0.11 and 0.97 µM, respectively [24]. Both of these compounds (**18–19**) were also active in the AChE inhibition assay with IC_{50} values of 32.5 and 43.0 µg/ml, respectively. Compounds **16** and **17** were found to be weakly active in the AChE inhibition assay, while they were inactive in the GST inhibition assay [25].

(**16**)

(**17**)

(**18**)

(**19**)
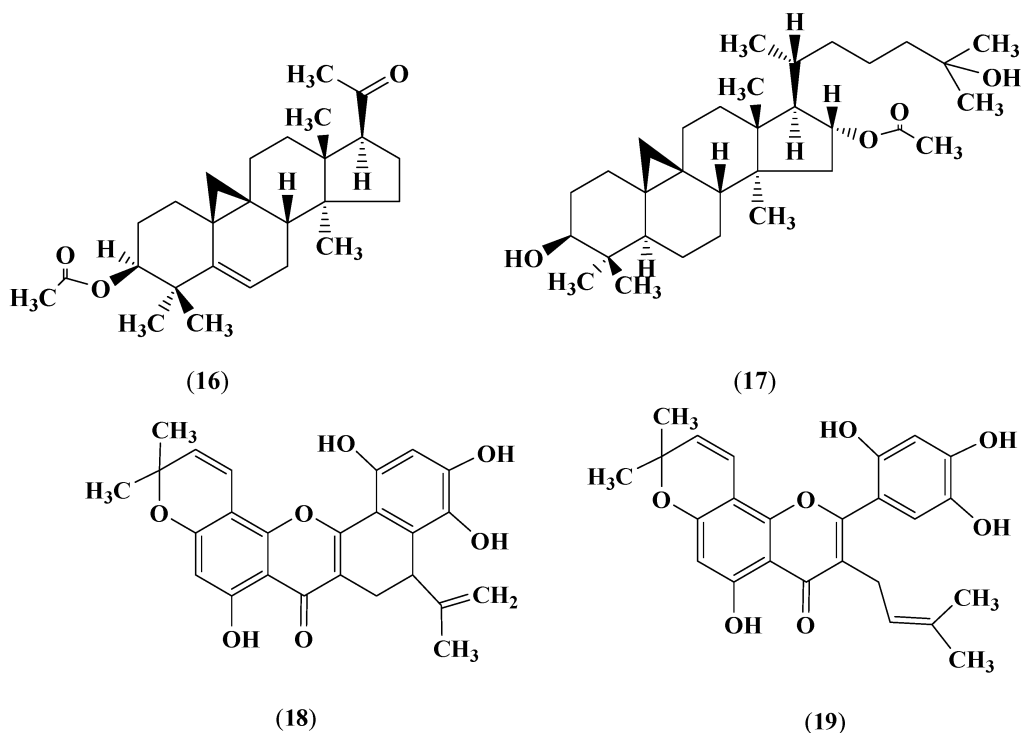
AChE-INHIBITING STEROIDAL ALKALOIDS FROM *BUXUS HYRCANA*

Buxus alkaloids have a unique steroid-triterpenoid pregnane-type skeleton with C-4 methyls, and 9β,10β-cycloartenol system with a degraded C-20 side chain [26]. These alkaloids exhibit various biological activities, including anti-HIV, anti-TB, antimalarial, and enzyme inhibitory activities, etc. [27]. Previous chemical studies on various species of genus *Buxus* have resulted in the isolation of over 200 new steroidal alkaloids [9]. *Buxus hyrcana* is abundant in Iran, and previously, over 10 new steroidal alkaloids have been reported from this plant [28].

The crude methanolic extract of *B. hyrcana* exhibited AChE inhibitory activity in our bioassay with an IC$_{50}$ value of 45.0 µg/ml. Our detailed phytochemical studies on the bioactive fraction of *B. hyrcana*, collected from Iran, resulted in the isolation of two new steroidal alkaloids, O^6-buxafurandiene (**20**) and 7-deoxy-O^6-buxafurandiene (**21**) along with four known steroidal bases, benzoylbuxidienine (**22**), buxapapillinine (**23**), buxaquamarine (**24**), and irehine (**25**). Compounds **20–25** were tested for AChE inhibition activity, and it was found that compounds **20** and **21** exhibited AChE inhibition activity with IC$_{50}$ values of 17.0 and 13.0 µM, respectively. Compounds **22–25** showed moderate enzyme inhibition activity with IC$_{50}$ values of 35.0, 80.0, 76.0, and 100.0 µM, respectively. This bioactivity data suggests that the higher enzyme inhibition potency of compounds **20** and **21** might be due to the presence of a tetrahydrofuran ring in these compounds [28]. Furthermore, compounds **20** and **21** exhibited nearly the same bioactivity and this further suggested to us that the C-7 hydroxyl group does not play any role in the bioactivity. Compounds **20** and **21** belong to the rarely occurring class of *Buxus* alkaloids having a tetrahydrofuran ring incorporated in their structures.

(**20**) R = OH
(**21**) R = H

(**22**) R = PhCO

(**23**) R = PhCO

(**24**)

(**25**)
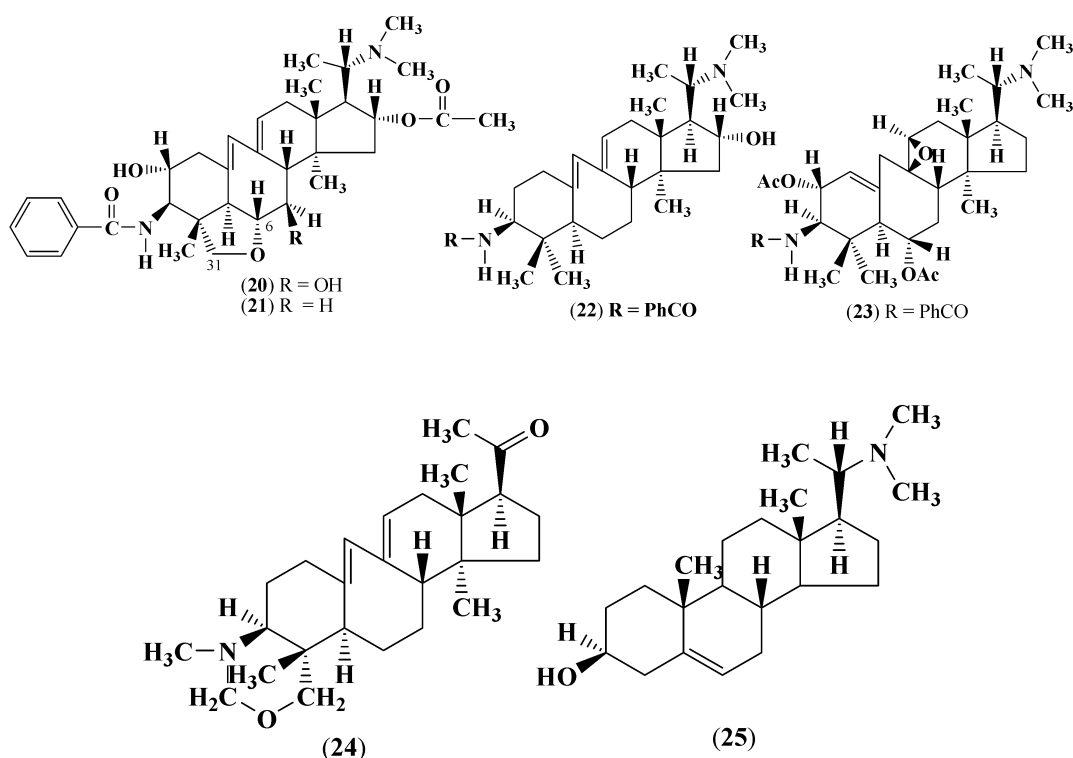
MUCORALACTONE A: A NOVEL STEROIDAL AChE INHIBITOR FROM *MUCOR PLUMBEUS*

A number of natural products having potential biomedical applications have been isolated from microorganisms. For instance, cyclosporine A FK506 and rapamycin are used as immunosuppressants [29]. In our initial screening, we discovered that the crude ethyl acetate extract of *Mucor plumbeus* exhibited AChE inhibitory activity with an IC$_{50}$ value of 30 µg/ml. Our detailed chromatographic work on this crude extract resulted in the isolation of mucoralactone A (**26**), a novel steroid containing a lactone moeity incorporated in its structure. Compound **26** exhibited AChE inhibitory activity with an IC$_{50}$ value of 19 µg/ml [29]. It was also discovered that AChE activity of crude ethyl acetate extract of *M. plumbeus* was due to the presence of this compound.

(26)
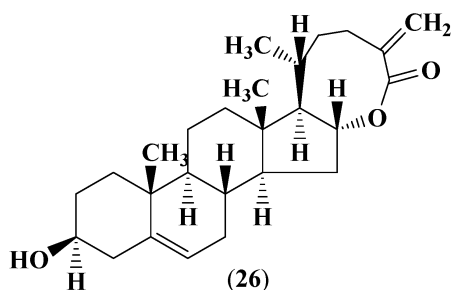
CONCLUSION

In summary, we have successfully identified two new iridoid glycosides (**5**) and (**6**) as potential GST inhibitors and a semi-synthetic analog of pipataline (**2**), 7-amino,8-hydroxypipataline (**8**), as an AChE inhibitor from *B. prionitis*. Chemical studies on *C. bonduc* afforded GST inhibiting 13,14-*seco*-steroids (**10–11**). Structural modification at C-3 or C-5 of these compounds resulted in a significant increase in their bioactivity. From *A. nobilis*, we have isolated two known flavonoids, artoblidxanthone (**18**) and artoninse (**19**) exhibiting a very promising GST inhibition activity. Chemical studies on the crude extracts of *B. hyrcana* and *M. plumbeus* afforded three novel compounds, O^6-buxafurandiene (**20**), 7-deoxy-O^6-buxafurandiene (**21**), and mucoralactone A (**26**). These three compounds exhibited significant AChE inhibitory activity. Further work on the SARs of these compounds is warranted.

ACKNOWLEDGMENTS

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