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Research article

Synthesis and biological activities of flavonoid sulfamates as steroid sulfatase inhibitors

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ABSTRACT

Synthesis of potent flavonoid sulfamate inhibitors of the enzyme steroid sulfatase (STS), a topical target in treating postmenopausal women with hormone-dependent breast cancer, is described in the current article. Novel compounds were examined for estrone sulfatase (E1-STS) inhibition in intact MCF-7 breast cells. The strategies adopted to develop STS inhibitors which, while active *in vivo*, are devoid of any estrogenicity that would limit their use for breast cancer therapy (e.g. estrone 3-*O*-sulfamate, EMATE), were broadly successful. Several molecules of flavonoid sulfamates were synthesized that potentially possess both aromatase and steroid sulfatase inhibitory properties. The isoflavane-4',7-*O*,*O*-bis-sulfamate (equol bis-sulfamate) and flavone-6,4'-*O*,*O*-bis-sulfamate were found to be the most potent inhibitors of all the flavonoid sulfamate analogs *in vitro*, inhibiting STS by about 99% at 0.1µM in MCF-7 cells. Some of these flavonoid sulfamates were also found to be active against both enzymes' STS and aromatase (e.g., 5-hydroxy-7-methoxyflavone-4'-*O*-sulfamate, 5-hydroxy-flavone-4',7-*O*,*O*-bis-sulfamate, and 5,7-dihydroxy flavanone-4'-*O*-sulfamate), thus demonstrating the novel concept of a dual inhibitor. The availability of flavonoid sulfamates as STS inhibitors may enable to use of them as a therapy in the treatment of breast cancer.

Keywords: Flavonoids, Sulfamates, Steroid sulfatase, Breast cancer, Inhibitors, Synthesis.

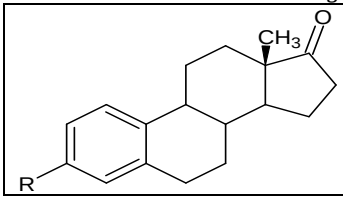
INTRODUCTION

Breast cancer is a significant health concern, and its incidence has increased globally. Breast cancer is a condition where malignant cells form in the breast tissues, and it affects women predominantly, although men can also develop it. Still, it's relatively rare in comparison. Various factors, including lifestyle choices, genetics, reproductive history, and environmental exposures, can influence the incidence of breast cancer. In high-income countries, such as the United States and many European nations, breast cancer has been particularly prevalent, affecting about one in eight women at some point [1-3]. This higher incidence rate in high-income countries can be attributed to several factors, including early detection through screening programs, changes in reproductive patterns, increased life expectancy, and lifestyle factors like obesity and sedentary behavior [4, 5]. Efforts to raise awareness about breast cancer, promote early detection through mammography and regular check-ups, and advance treatment options have been ongoing to reduce the disease burden and improve outcomes for affected individuals [6, 7]. Additionally, research into the underlying causes of breast cancer and potential risk factors continues to provide valuable insights into prevention and treatment strategies [8, 9].

Estrogens' influence on breast cancer development has long been recognized. It is generally believed that estrogens act as promoters rather than carcinogens in breast cancer development. Still, the underlying biological mechanism through which estrogens exert their effects remains poorly understood [10-12]. Steroid sulfatase (STS) is an enzyme that plays a crucial role in synthesizing estrogens. The synthesis of estrogens primarily occurs in the ovaries, placenta (during

pregnancy), and to a lesser extent, in the adrenal glands. Understanding the role of STS in estrogen synthesis is essential, as it represents a potential target for therapeutic interventions related to estrogen-related disorders. For instance, inhibiting the activity of this enzyme might be a strategy to control estrogen levels in conditions like hormone-dependent breast cancer or polycystic ovary syndrome [13-16]. Danazol was the first drug shown to have the ability to inhibit STS (62% at ten µM in MCF-7 cells). However, several steroidal and nonsteroidal drugs used to treat endocrine disorders have also been examined for their potential as steroid sulfatase inhibitors. Recognition of the pivotal role of steroid sulfatase in regulating the synthesis of biologically active steroids has led to the development of potent inhibitors [17-19]. Estrone-3-*O*-sulfamate (EMATE), and its *N*-methylated derivatives (Figure 1), were found to be potent STS inhibitors. [20]

Figure 1: Structures of EMATE and its N-methylated derivatives



	R
Oestrone 3-*O*-sulphamate (EMATE)	OSO_2NH_2
Oestrone 3-*O*- (*N*-methyl) sulphamate	OSO_2NHMe
Oestrone 3-*O*- (*N, N*-dimethyl) sulphamate	OSO_2NMe_2

In vitro, EMATE inhibits E1-STS activity by > 99% at 0.1 µM in intact MCF-7 breast cancer cells and in a time and concentration-dependent manner in a placental microsomes preparation, indicating that it acts as an irreversible inhibitor. However, EMATE was also shown to be a potent estrogen five times more estrogenic than ethinyloestradiol when administered orally to rats. Therefore, due to the sensitivity of endocrine-dependent tumors of the breast and endometrium to estrogens, EMATE is unsuitable for use as a sulfatase inhibitor in such diseases. Thus, due to the estrogenic activity of EMATE, there is a clear need for inhibitors, being metabolically stable, more selective, and devoid of estrogenic activity [20]. We focused on synthesizing several molecules with aromatase and steroid sulfatase inhibitory properties. Therefore, a range of flavonoid sulfamates was designed and synthesized based on comprehensive non-estrogenic templates to have compounds as potent as EMATE but with no uterine growth stimulating properties and, in some cases, possibly also possessing intrinsic aromatase inhibitory properties.

Flavonoids (flavone, isoflavone, and flavanone) are ubiquitous secondary plant metabolites, and a regular human diet will likely contain about one gram per day of these natural compounds [21]. Natural or synthetic flavonoids are known to exhibit various biological activities. In particular, many combinations indicate antitumor or related activities, such as antimitotic activity, topoisomerase, protein kinase C, several protein-tyrosine kinases, or cyclin-dependent kinases. Flavonoids have also been shown to possess anti-inflammatory, anti-allergic, antiviral, anti-mutagenic, and anti-carcinogenic activities. Furthermore, some of these compounds were found to have estrogenic or antiestrogenic activities and aromatase inhibitory activity. The vast majority of flavonoids have low toxicity to humans and animals [22]. The low incidence rate of breast cancer in the Far East (rather than genetic difference being an essential factor) has been related to a high dietary intake of soya products containing flavones and isoflavones. Genistein, a flavonoid derivative, is commonly found in soya products.

This compound has been reported to bind to the estrogen receptor, inhibit tyrosine protein kinase, and have anti-angiogenic and anti-proliferative activity. The low rates of incidence of breast cancer among Japanese and Chinese are due to their high intake of soya products, which may be explained by the inhibition of aromatase and/ 17β-hydroxysteroid dehydrogenase as it has been shown that some flavonoids present in soya products inhibit these enzymes [23-26].

For years considerable research has been carried out to develop potent aromatase inhibitors, some of which are clinically in use. However, in reports of postmenopausal women with breast cancer who received aromatase inhibitor therapy, plasma estrone sulfate (E1S) concentrations remained between 400-1000 pg/ml, which is high. To block not only the synthesis of estrone from androstenedione but also block E1S and the formation of androstenediol from dehydroepiandrosterone sulfate (DHA-S), there may be a considerable therapeutic advantage to be achieved by treating such women with a combination of aromatase and sulfatase or even with a dual sulfatase/aromatase inhibitor if such a molecule could be developed [27-29]. We have now explored the concepts of using flavonoids and related molecules as templates to design nonsteroidal sulfatase inhibitors and also the idea of inhibiting sulfatase and aromatase activities with a single inhibitor by synthesizing a series of flavonoid (flavone I, isoflavone II, flavanone III and isoflavane) sulfamates (Table 1, Figures 2-4 and 1-26).

MATERIALS AND METHODS

General Methods

All reagents and solvents employed were purchased from Aldrich, Sigma Chemicals, or Lancaster Synthesis. Silica gel refers to silica gel, Merck, grade 60. Product(s) and starting material were detected by viewing under ultraviolet (UV) light or treating with a methanolic solution of phosphomolybdic acid followed by heating. NMR spectra were determined using Acetone-d_6, Deuterated chloroform ($CDCl_3$), or Dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent and Tetramethylsilane (TMS) as an internal standard. The proton nuclear

magnetic resonance (^{1}H NMR) and Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Jeol GX 270 at 270 MHz and a Jeol EX 400 at 400 MHz NMR spectrometer. The following abbreviations are used to describe resonances in ^{1}H NMR and ^{13}C NMR spectra: s, singlet; d, doublet; br, broad; t, triplet; q, quartet; m, multiplet, and combination such as dd, doublet of doublets. Infra-Red (IR) spectra were determined as KBr discs using a Perkin-Elmer 782 Infra-Red Spectrophotometer. Melting points were determined on a Reichert-Jung Kofler Block and were uncorrected. Mass spectra were recorded on VG 7070 and VG Autospec instruments at the Mass Spectrometry Service at the University of Bath. Fast atom bombardment (FAB) mass spectra were carried out using m-nitrobenzyl alcohol (m-NBA) as the matrix. CHN analysis was determined using gas chromatography at the Microanalysis Service at the University of Bath. All reagents and solvents were stored away from moisture and light and dried before use. Low-temperature experiments were conducted using a well-insulated external bath containing either ice/water with NaCl for 0°C or carbon dioxide pellets with acetone or a cold plate. Experiments requiring anhydrous conditions were guarded using a drying tube containing self-indicating silica. Evaporation of solvents was carried out with a rotary evaporator at reduced pressure (water pump) and, on stated occasions, followed by a high vacuum pump. Samples were dried under a high vacuum and low temperature in a drying tube.

Biological Assay of Sulfamates

All assays were performed at the Department of Endocrinology and Metabolic Medicine, Imperial College School of Medicine, St. Mary's Hospital, London.

Sulfatase Inhibitor:

Sulfatase inhibition was assessed using intact MCF-7 breast cancer cells as described by Purohit *et al.* [20].

Aromatase Inhibitor:

Placental microsomes were also used to assess the aromatase inhibitory properties of flavonoid sulfamates using a tritiated water release assay [20]. Placental microsomes (200 µL) were incubated with [1β-^3H] androstenedione, 60 µM and 1µM and 1µM NADPH in the absence or presence of an inhibitor.

Preparation of Sulfamoyl Chloride

Sulfamoyl chloride was prepared by the reaction of chlorosulfonyl isocyanate with formic acid according to the method of Appel and Berger (Scheme 1) [30].

Scheme 1: Preparation of Sulfamoyl Chloride, (i) Toluene (anhydrous-free sulfur impurities), 0°C-RT, under N_2, 12 hours.

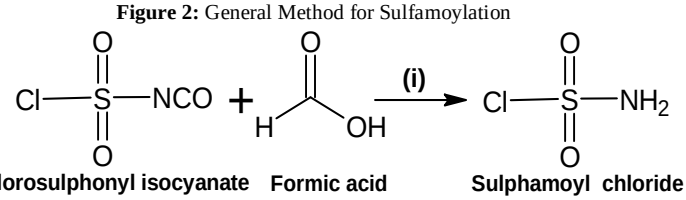
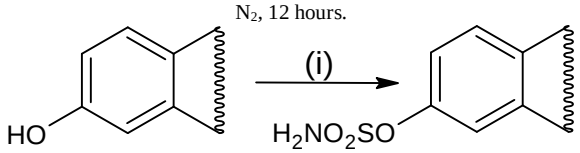


Figure 2: General Method for Sulfamoylation

Starting with the parent compound, the sulfamate derivatives were prepared as previously described [20], unless stated otherwise; in this regard, a solution of the appropriate parent compound in anhydrous DMF was treated with sodium hydride [60% dispersion; 1.2 and 2.5 equiv. For monohydroxyl and dihydroxyl compounds, respectively, unless stated otherwise] at 0°C under an atmosphere of N_2. After hydrogen evolution had ceased, sulfamoyl chloride in toluene [excess, ca. 5- 6 eq.] was added (Scheme 2), the reaction mixture was poured into brine after warming to room temperature overnight, and ethyl acetate was added. The organic fraction was washed exhaustively with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The crude product obtained was purified by flash chromatography or preparative TLC, followed by recrystallization to give the corresponding sulfamate. All the compounds were characterized by spectroscopic and combustion analysis.

Scheme 2: Synthesis of sulphamates, (i) NaH/DMF, $ClSO_2NH_2$, 0°C-RT, under N_2, 12 hours.



RESULTS AND DISCUSSION

Synthesis

The sulfamoyl group has been widely utilized as an activity-modifying substituent in several different classes of drugs [31, 32]. For example, N-substituted steroidal sulfamates have been synthesized, including sulfamates of estradiol, to block metabolic conjugation, but their ability to inhibit El-STS was not explored. In most of the earlier works of this group (e.g., EMATE), the sulfamoylation of phenolic compounds was carried out directly with crude residue/crystalline sulfamoyl chloride, prepared according to the method of Appel and Berger. However, the continual handling of sulfamoyl chloride in the solid state proved cumbersome since this reagent is highly hygroscopic and decomposes readily even on storage at low temperatures. Nevertheless, it was found that these drawbacks could largely be surmounted if the freshly prepared crude sulfamoyl chloride was stored instead as a solution in anhydrous and sulfur impurities-free toluene under nitrogen [33]. Flavonoid sulfamates (Table 1, Figures 2-4 and 1-26) were prepared analogously to other sulfamates described previously; for example, sulfamoylation of 4', 7-dihydroxy isoflavone (Diadzein) gave isoflavone-4',7-*O*,*O*-bis-sulfamate (Figure 3, 22) (Scheme 3). Briefly, a solution of the appropriate flavone, isoflavone, or flavanone in anhydrous DMF was treated with sodium hydride (1.2 and 2.5 equiv. for monohydroxy and dihydroxy flavonoids, respectively) at 0°C under N_2, then sulfamoyl chloride in toluene (3-5 equiv.) was added. After the mixture had warmed to room temperature overnight, the reaction was quenched with ethyl acetate and ice/water. After a workup for each flavonoid sulfamate, the crude product was purified by flash chromatography and recrystallization. All compounds

were fully characterized by spectroscopic and combustion analysis. The yield of the sulfamates was generally improved significantly by using excess sulfamoyl chloride (> 5 eq.) [20, 31].

Commercially unavailable (±) equol (Figure 4, 25) was prepared by catalytic hydrogenation of daidzein (Scheme 3) in a diglyme/acetic acid mixture using Pd-C.200 The Pd-C catalyst was first suspended in glacial acetic acid and shaken in an oxygen atmosphere for three days. The hydrogenation was rapid, and the conversion into (±) equol was complete within 30 minutes. However, the disadvantage of this method is that a large amount of catalyst is required; in attempts to use less motivation, the hydrogenation was found to be very slow and give a mixture of products. Flavone-7-methane sulfonate (Table 1, 17) was prepared by reacting 7-hydroxyflavone with methane sulfonyl chloride in pyridine.

I

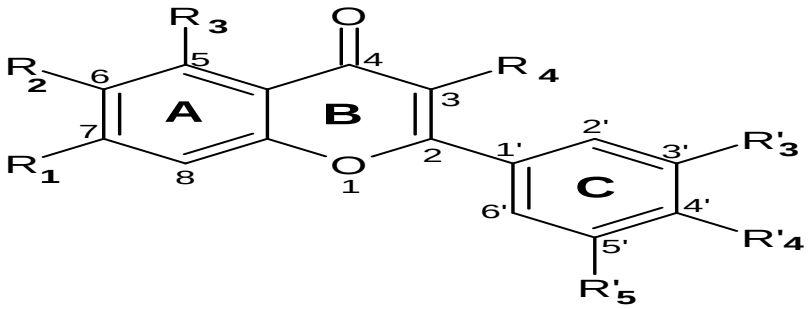
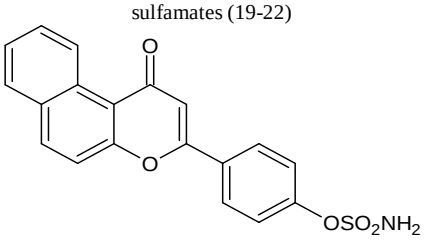


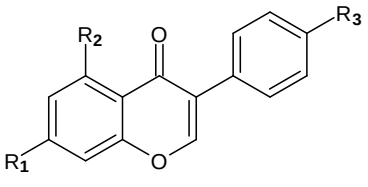
Table 1: Structures of 7-hydroxy flavone (ii), 6-hydroxy flavone (iv), 5-hydroxy-7- methoxy flavone (v), flavone sulfamates (1-16), and flavone-7-methane sulfonate (17).

	R1	R2	R3	R4	R'3	R'4	R'5
(i)		H	H	H	H	H	H
(ii)	OH	H	H	H	H	H	H
(iii)	H	OSO_2NH_2	H	H	H	H	H
(iv)	H	OH	H	H	H	H	H
(v)	OCH_3	H	OH	H	H	H	H
[1]	OSO_2NH_2	H	OH	H	H	H	H
[2]	OCH_3	OSO_2NH_2	OH	H	H	H	H
[3]	OCH_3	H	OH	H	H	OSO_2NH_2	H
[4]	OCH_3	OCH_3	OH	H	OSO_2NH_2	OCH_3	H
[5]	OCH_3	OCH_3	OCH3	H	OSO_2NH_2	OCH_3	H
[6]	H	H	H	H	OSO_2NH_2	OSO_2NH_2	H
[7]	OSO_2NH_2	H	OH	H	H	OSO_2NH_2	H
[8]	OSO_2NH_2	H	OH	OSO_2NH_2	H	H	H
[9]	OSO_2NH_2	H	OH	OSO_2NH_2	H	OCH_3	H
[10]	OSO_2NH_2	H	OH	OSO_2NH_2	OCH_3	OCH_3	OCH_3
[11]	OH	H	OH	OSO_2NH_2	OCH_3	OCH_3	OCH_3
[12]	OSO_2NH_2	H	H	OSO_2NH_2	OCH_3	OCH_3	OCH_3
[13]	OH	H	H	OSO_2NH_2	OCH_3	OCH_3	OCH_3
[14]	OH	OSO_2NH_2	OH	H	H	H	H
[15]	H	OSO_2NH_2	H	H	H	OSO_2NH_2	H
[16]	H	H	H	H	H	OSO_2NH_2	H
[17]	OSO_2CH_3	H	H	H	H	H	H

Figure 2: Structures of β-naphthoflavone sulfamates (18) and isoflavone sulfamates (19-22)

18

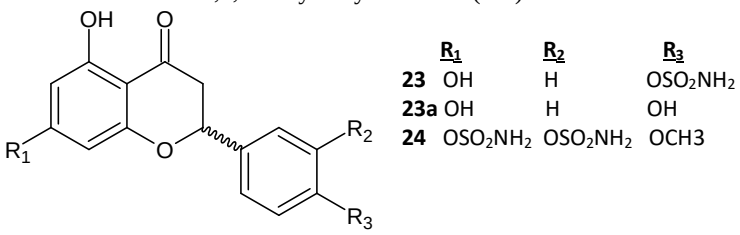




	R1	R2	R3
19	OSO_2NH_2	OH	OCH_3
20	OSO_2NH_2	OH	OSO_2NH_2
21	OH	OH	OSO_2NH_2
22	OSO_2NH_2	H	OSO_2NH_2

II

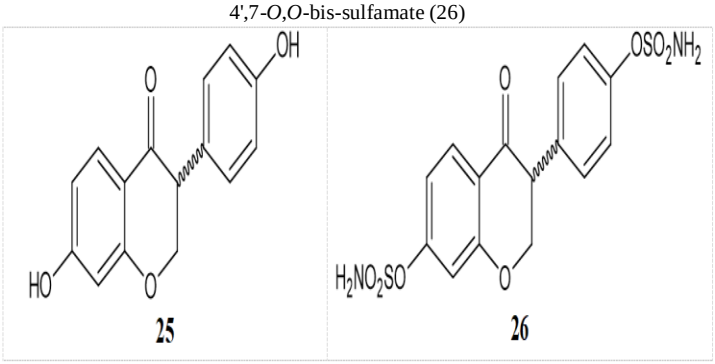
Figure 3: Structures of (±) flavanone sulfamates (23 and 24) and (±) 4',5,7- trihydroxy flavanone (23a)



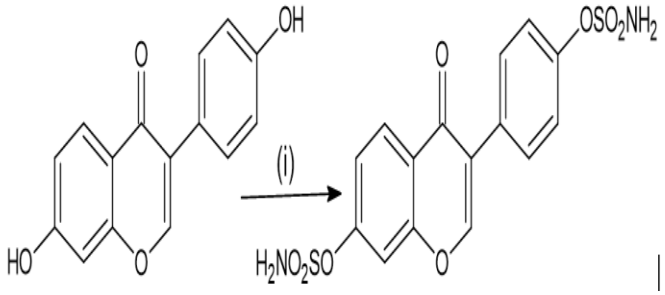
	R1	R2	R3
23	OH	H	OSO_2NH_2
23a	OH	H	OH
24	OSO_2NH_2	OSO_2NH_2	OCH3

III

Figure 4: Structures of (±) 4',7-dihydroxy isoflavane (25) and (±) isoflavane-4',7-*O*,*O*-bis-sulfamate (26)



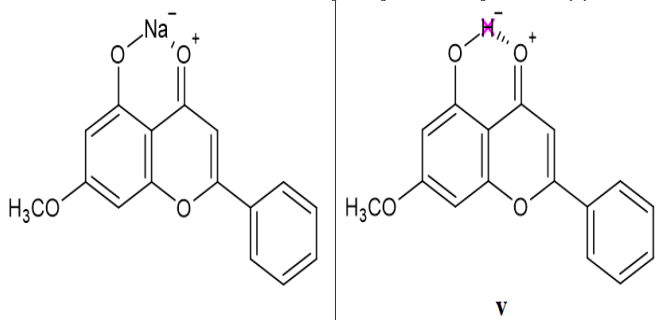
Scheme 3: Synthesis of isoflavone-4',7-*O*,*O*-bis-sulfamate (22), Reagents: (i) NaH/DMF, $ClSO_2NH_2$, 0°C-RT, 12 hours.



Diadzein **22**

A hydroxyl group at the 5-position of the flavonoids proved impossible to sulfamoylate even by using an excess of NaH. It was confirmed by attempting to sulfamoylate 5-hydroxy-7-methoxy flavone (Table 1, v). The reaction failed, and a plausible explanation is the formation of a complex between the sodium and the ketone group occurring at the 4-position of the flavonoid, followed by the appearance of an intramolecular hydrogen bond when the reaction was quenched (Figure 5), as shown by ^{1}H NMR, the proton of the 5-position hydroxyl group was recorded at about 11-12 ppm (downfield).

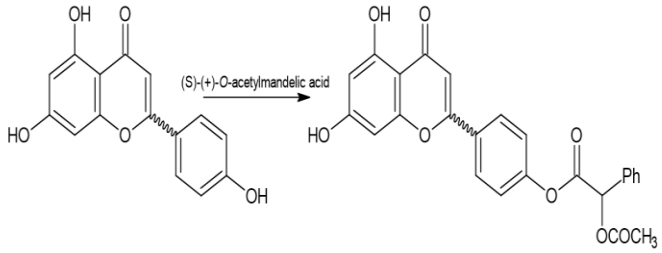
Figure 5: The formation of a complex and the hydrogen bond between the C-5-OH and the C-4-C=O of 5-hydroxy-7-methoxy flavone (v)



V

Only the monosulfamate (Figure 3, 23) could be obtained from (Figure 3, 23a) even using 2.5 eq. of NaH; the bis-sulfamate was either not formed or degraded due to instability. The resolution of the enantiomers of 5,7,4'-trihydroxy flavanone (23a) to determine the potentially active isomer after sulfamoylation was attempted using (S)-(+)-*O*-acetyl mandelic acid (Scheme 4). The 4'-acetyl mandelic acid derivative of 23a (Scheme 4; I) was obtained, and no separation of diastereoisomers resulted. The chiral center (C-2) is far from C-4' and thus has no influence.

Scheme 4: 4'-Acetylmandelic acid derivative of 5,7,4'-trihydroxy flavanone



23a **I**

Biological Data

Inhibition of El-STS in MCF-7 breast cancer cells by flavone (1-18), isoflavone (19-22), and flavanone (22-24) sulfphamates are shown in Tables 2 and 3. The inhibition of aromatase in placental microsomes by most flavonoid sulfamates is also included. The inhibitory activity of (±) equol bis-sulfamate 26 is also included in Table 3. These compounds significantly inhibit E1-STS in MCF-7 cells. Compounds 15, 20, 22, 23, and 26 are the most active in this series, with inhibitory activity of about 99% in MCF-7 cells at ten µM. Generally, it was found that the sulfamates of isoflavone and isoflavane, i.e. 26, are more potent than flavone and flavanone sulfamates as steroid sulfatase inhibitors. It was also found that bis-sulfamates are more powerful than the corresponding monosulfamate (e.g., 20 and 21). The effect of a sulfamate group when attached to a non-aromatic ring (in this case, ring C of the flavonoids) was also evaluated, and the products were found to be inactive (e.g. 11 and 13), indicating again, as we expect, that the phenyl ring structure is required for potent inhibition. In general, the aromatase inhibitory activity of flavone sulfamates (e.g. 1, 3, 4, and 7, Table 2) was found to be more marked than flavanone sulfamates (23 and 24, Table 3) and isoflavone sulfamates (19-22, Table 2), which were found to be inactive.

The parent phenolic compounds of these sulfamates were all found to be inactive for inhibition of E1-STS, which indicated that the sulfamate group is the core motif of these molecules' recession to exhibit sulfatase inhibitory activity. In contrast, the aromatase inhibitory activity for some of these phenol parent compounds was evaluated in placental microsomes (e.g. Table 1; ii and iv). In these two examples, the sulfamoyl group (i and iii) slightly reduces the aromatase inhibitory activity compared to the phenol parent compounds (ii and iv). 7-Hydroxyflavone (ii) inhibited aromatase activity by 96% at ten µM in

placental microsomes, while flavone-7-*O*-sulfamate (i) inhibited aromatase activity by 86 % at the same concentration (Table 2). Other parent phenol compounds of these sulfamates were not evaluated for aromatase inhibitory activity as other groups do them [24, 25]. The inhibitory activity of their sulfamates was compared with the results in the literature. Introducing the sulfamoyl group to these flavonoids did not influence the aromatase inhibitory activity.

It has been demonstrated that EMATE is a potent irreversible inhibitor of E1-STS. However, since EMATE is highly estrogenic, there is a need to develop other classes of potential inhibitors [20, 34]. Having identified the critical chemical structural requirement for potent sulfatase inhibition (i.e. phenolic ring structure and -*O*-sulfamoyl group), we considered that the introduction of the sulfamoyl group into the flavonoid structure might significantly engender sulfatase inhibitory activity and, also might provide new leads for the design of a dual nonsteroidal sulfatase/aromatase inhibitor, as several flavonoids have aromatase inhibitory activity [24, 25, 34, 35]. Initially, 7-hydroxy-flavone ii (a known potent aromatase inhibitor, which inhibits this enzyme by 96% in placental microsomes at 10µM) and 6-hydroxy flavone iv (inhibits aromatase by 30% in placental microsomes at 10µM) were sulfamoylated to give flavone-7-*O*-sulfamate i and flavone-6 -*O*-sulfamate iii respectively. Flavone-7-*O*-sulfamate (i) was found to inhibit aromatase in placental microsomes by 8 6 % at 10µM but was found to be inactive against E1-STS in MCF-7 cells at 0.1-10 µM, while flavone-6-*O*-sulfamate iii, was found to be inactive against aromatase, but found to inhibit E1-STS activity in MCF-7 cells by 99% at 1µM and 10µM. These results were encouraging and supported synthesizing flavonoid sulfamates, which may lead to the eventual development of dual aromatase/sulfatase inhibitors. Therefore, several flavonoid sulfamates have been designed and synthesized here (1-26) [24].

Biological evaluation of the novel synthetic entities revealed that most of these flavonoid sulfamates (1-26) show potent sulfatase inhibition at 10µM in MCF-7 breast cells. Also, some of them offer pretty potent aromatase inhibition at 10µM in placental microsomes (Tables 2 and 3). Compounds (19, 20, 21, 22, and 23) inhibited E1-STS in MCF-7 cells in ranges of 23- 87% at 0.1 µM, 79-99% at one µM, and > 99% at ten µM. One of the best inhibitors in this series, isoflavone 4',7-*O*,*O*-bis-sulfamate (22), inhibited E1-STS activity in MCF-7 cells by 87% and > 99% at 0.1 µM and one µM respectively, but no aromatase inhibition activity was detected (Table 3). The high potency of 22 might be due to its similarity to the structure of EMATE, as shown by a computer modeling study (Figure 6). The ring structure of this compound presumably mimics the steroid rings (A, B, and D) of EMATE, and might have similar hydrophobicity of EMATE, which is required for extra binding to the enzyme and thus exhibits potent E1-STS activity. However, it might also act by the exact mechanism as EMATE, but we do not know if either one or both sulfamate group (s) are involved in the sulfamoylation of the enzyme.

Table 2: Inhibition of E1-STS and aromatase activity by flavone sulfamates (1-16), flavone-7-methane sulfonate (17) and β-naphthoflavone 4'-*O*-sulfamate (18), - = not determined, *= inactive (< 1 0 %), ♣ = solubility problem, ♥ = results not yet known, negative sign of %inhibition = stimulation effect.

	% Inhibition Sulphatase MCF-7 Cells			Aromatase
	0.1µM	1µM	10µM	10µM
i	*	*	*	86
ii	-	-	-	96
iii	-	99±0.6	98.9±1.3	*
iv	-	-	-	30
[1]	-	*	20.6±1.2	76
[2]	11±2.7	51±3	78±1.7	*
[3]	3.5±4	88±3.1	99±0.6	85
[4]	*	18±6.1	27±3.5	93
[5]	29±0.8	23±1.8	12±0.5	*
[6]	*	79±0.8	29±1.3	*
[7]	*		98±0.4	89
[8]	*	*	12±1.5	13
[9]	*	*	*	*
[10]	*	*	*	35
[11]	*	*	*	34
[12]	*	*	*	22
[13]	*	*	*	32
[14]	18±0.8	42±1.3	95±0.3	♥
[15]	>99	>99	>99	♥
[16]	15±0.7	65±1.2	96±0.2	♥
[17]	*	*	*	♥
[18]	13±5.9	77±1.2	95±0.1	♥

Table 3: Inhibition of E1-STS and aromatase activity by isoflavone sulfamates (19- 22), flavanone sulfamates (23 and 24), and isoflavane sulfamate (26). - = not determined, *= inactive (< 1 0 %).

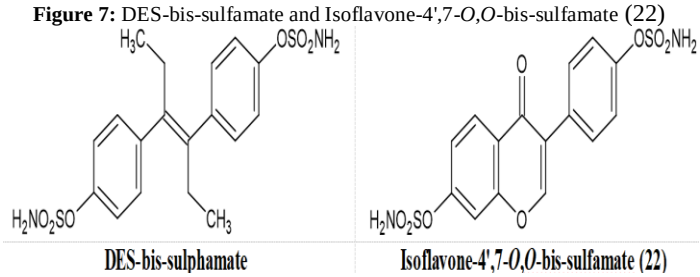
	% Inhibition Sulphatase MCF-7 Cells			Aromatase
	0.1µM	1µM	10µM	10µM
[19]	*	11±3.0	37±1.7	*
[20]	28±1.7	90±0.6	99±1.4	*
[21]	23±2.2	83±2.7	99±0.7	*
[22]	87±2.5	>99	>99	*
[23]	30±2.8	79±1.2	98±1.2	85
[24]	2±1.3	8±0.6	9±1.2	63
[25]	98±0.8	>99	>99	13

The spacing between the two phenyl rings of isoflavone sulfamates, such as 22, has a certain degree of similarity to that of the diphenylethylene-type estrogen (diethylstilboestrol, DES), whose estrogenicity has been attributed to a structural mimicry of estradiol. Since DES-bis-sulfamate (Figure 7) is a potent E1-STS inhibitor (IC_{50}

10µM in intact MCF-7 breast cells), the high potency observed for 22 could be the result of a similar mimicry of the steroid nucleus of EMATE. Indeed, a computer modeling of EMATE and isoflavone 4',7-*O*,*O*-bis-sulfamate 22, as shown in (Figure 6) supports such an argument [34].

Figure 6: A computer modeling of EMATE and isoflavone-4',7-*O*,*O*-bis-sulfamate (22)

Figure 7: DES-bis-sulfamate and Isoflavone-4',7-*O*,*O*-bis-sulfamate (22)



The isoflavone sulfamates 19-22 and isoflavone bis-sulfamate 26 were found to be potent E1-STS inhibitors but, disappointingly, were found to be inactive or have only poor inhibitory activity against aromatase in placental microsomes at 10µM (Table 3). In contrast, the corresponding flavone sulfamates were found to be more potent aromatase inhibitors (e.g., 7). The difference in aromatase inhibition by such sulfamates might be due to binding affinity to the active site of the enzyme, and flavone sulfamates are likely better at binding to the aromatase active site than isoflavone or isoflavone sulfamates. This observation was similar for the parent phenol compounds; isoflavones are weaker aromatase inhibitors than flavones. It has been postulated that the C-4 carbonyl group of flavones such as ii coordinate with the heme moiety of aromatase similarly to aminoglutethimide, exhibiting inhibitory activity against the enzyme. For isoflavone compounds, such as carbonyl group and heme iron interaction may be made ineffectual by the aromatic via steric hindrance B-ring at the C-3 position. For isoflavane compounds, such interaction does not occur due to the absence of the C-4 carbonyl group. It was also found that flavone-6-*O*-sulfamate (iii) is a significantly weaker aromatase inhibitor than flavone-7-*O*-sulfamate (i). This finding indicates that the region around the C-6 position in the active site of aromatase might be very restricted or ineffective via steric hindrance by the aromatic ring [24, 25].

Compounds 3, 4, 7, and 23 inhibited aromatase activity by 85%, 93%, 89%, and 85% at 10 µM in placental microsomes, respectively (Tables 2 & 3). These compounds also inhibited E1-STS in MCF-7 cells by 99%, 27%, 98%, and 98% at ten µM. The results of these compounds prove our novel concept that it is possible to inhibit both enzymes (E1-STS and aromatase) by a potential dual inhibitor. However, it is desirable to establish whether the inhibition of aromatase by these compounds is an inherent property of the molecule or is the result of an *in vivo* activation of the parent phenols since some of them are known potent aromatase inhibitors (e.g., 7-hydroxy-flavone, 4',5,7-trihydroxy-flavone). Therefore, flavone-3',4'-*O*,*O*-bis-sulfamate (6), flavone-4'-*O*-sulfamate (16), and flavone-7-methanesulfonate (17) were synthesized and evaluated for their ability to inhibit E1-STS and aromatase activity. Compound 6 was found to be a weak E1-STS inhibitor (inhibited E1-STS, 29% at 10µM in MCF-7 cells). This weak inhibition may be because the second sulfamate group (at C-3') may have caused the other sulfamate group at C-4' to be shifted in the binding site from the usual position occupied by the sulfamate group of those active compounds which have an unhindered sulfamoyl group at C-4' (e.g. 3 and 7). Hence, it could not be activated effectively for the sulfamoylation of the enzyme. It may also be the reason for poor inhibition when a substituent such as methoxy(s) was introduced at the flavonoid sulfamates rings (e.g. 2, 4, 5, 9, 10, and 11).

These results have therefore highlighted the limited tolerance of the enzyme to substituents. It is also possible that the methoxy(s) or the second sulfamate group may conceivably shield the sulfamate group in such a manner that the putative proton abstraction, the first vital step proposed for the mechanism of E1-STS inhibition by EMATE, is prevented from occurring. Compound 16 was a potent E1-STS inhibitor (inhibited E1-STS activity by 96% at 10µM in MCF-7 cells), and compound 17 was inactive against E1-STS (Table 2). We expect compounds 16 and 17 to be fixed against aromatase (results not yet known). If our expectation is correct, this demonstrates that the inhibition of aromatase activity results from the flavonoid sulfamate and is not due to an *in vivo* activation of the parent phenol compounds. The β-naphthoflavone compounds were found to be relatively weak or inactive aromatase inhibitors because their structure does not fit in the enzyme's active site. However, for further confirmation of the concept, 4'-hydroxy-β-naphthoflavone has been sulfamoylated to give β-naphthoflavone 4'-*O*-sulfamate 18, which was evaluated for estrone sulfatase inhibitory activity. This compound was found to be a potent sulfatase inhibitor (inhibited E1-STS activity by 95% in MCF-7 cells at 10µM), and it is expected to be a relatively potent aromatase inhibitor and slightly better

than its parent starting material (results not yet known). If our speculation is correct, compound 18 and other flavonoid sulfamates are possibly dual inhibitors. But in the case of unstable flavonoid sulfamates (e.g., flavone 7-*O*-sulfamate) or after sulfamoylation of the enzyme, which releases the parent phenol compounds, these sulfamates might act as pro-drugs [24, 25].

As we expected that the 7-hydroxy or sulfamoyl group at 7-position, or 4' was required for potent aromatase inhibitory activity (e.g. i, ii, 4, 18, and 23) and the sulfamoyl group at the 4' or six positions of the flavonoid structure is required for potent sulfatase inhibitory activity. Further modifications have also been carried out in this series which significantly improve sulfatase and aromatase inhibitory activities, e.g. flavone-5,7-dihydroxy-6-*O*-sulfamate 14, and flavone-4',6-*O*,*O*-bis-sulfamate (15). Compound 15 shows potent E1-STS inhibitory activity (inhibited E1-STS activity by > 99% at 0.1 µM in MCF-7 cells (Table 2) and is expected to have a moderate aromatase activity (results not yet known). In contrast, 14 is expected to show potent inhibitory activities against both enzymes (inhibited E1-STS activity in MCF-7 cells by 96% at 10µM). Flavone-4',6-*O*,*O*-bis-sulfamate 15 were the most potent E1-STS inhibitors in these series (Table 2).

Overall, this study has revealed that flavonoid sulfamates are a new series of potential E1-STS inhibitors that can significantly inhibit E1-STS activity and aromatase activity. We have also provided preliminary evidence supporting the novel concept of a dual sulfatase/aromatase inhibitor based on the flavonoid system. However, more work is required to explore this goal thoroughly. Still, some of the compounds reported here should prove to be valuable new leads, either alone (e.g., 3, 7, 16, 24) or as a combination of a potent El-STS inhibitor (iii, 14, 15, 18, 20, 21, 22, 26) and potent aromatase inhibition (e.g. i, ii or 4). Inhibition of steroid sulfatase is an essential target for developing new drugs for treating women with endocrine-dependent breast tumors. The first potent sulfatase inhibitor identified EMATE proved, unexpectedly, to be estrogenic. Therefore, several strategies have been adopted to design and synthesize non-estrogenic inhibitors and explore further structure-activity relationships for sulfamate-based steroid sulfatase inhibitors. The attempts to overcome the estrogenicity of EMATE were broadly successful. This led to the synthesis of potent E1-STS inhibitors, which were devoid of any estrogenicity. Some of these compounds were found to possess both aromatase and steroid sulfatase inhibitory properties potentially. Considerable progress has been made in developing several potent nonsteroidal sulfatase inhibitors; In contrast, these inhibitors include greater potency, but their ability to inhibit sulfatase activity was still relatively modest compared to estrone 3-*O*-sulfamate.

CONCLUSION

This study has demonstrated that the sulfamoyl group is essential for E1-STS inactivation, as most of these flavonoid sulfamates are potent and significantly inhibit E1-STS activity in MCF-7 cells of 10 µM or lower. Our data also indicate that flavonoid sulfamates (3, 4, 7, and 23) are potent sulfatase inhibitors with potential aromatase inhibitory activity. Therefore, these compounds represent essential lead compounds for optimizing potential dual sulfatase/aromatase inhibitors. Flavonoid sulfamates may be effective therapeutic agents in treating estrogen-dependent breast cancer. These results will also lead to further studies to design and maximize the activity of metabolically stable potent dual sulfatase/aromatase inhibitors and to make the compounds completely devoid of estrogenic activity, as it is known that some flavonoids are estrogenic at high doses [36].

EXPERIMENTAL SECTION

Synthesis of Flavonoid Sulfamates

5-Hydroxyflavone-7-*O*-sulfamate (1) Upon sulfamoylation, 5,7-dihydroxyflavone (1.0 g, 3.933 mmol) gave the crude product (1.13g) which was fractionated by flash chromatography (chloroform/acetone, 8:1). The yellow residue that was obtained (324 mg, 24%) was further purified by recrystallization from ethyl acetate/hexane (1:1) to give **1** as yellow crystals (213 mg, 16%); mp 195-200 °C (dec.); TLC (chloroform/acetone, 12:1, 8:1 and 4:1): R*f*s 0.21, 0.25 and 0.44 respectively; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3360 (NH_2), 1650 (C=O), 1380 ($-SO_2$) cm^{-1}; δ_H (270 MHz, acetone-d_6) 6.75, 6.98 and 7.17 (3H, three s, C-3-H, C-6-H, and C-8-H), 7.63 (2H, br s, exchanged with D_2O,-OSO_2NH_2), 7.65 (3H, m, C-3'-H, C-4'-H, and C5'-H), 8.15 (2H, d, *J*= 7.7 Hz, C-2'-H and C-6'-H) and 13.0 (1H, br s, exchanged with D_2O, C-5-OH); MS. m/z (FAB+) 334.1 [100, $(M+H)^+$], 255.0 [25, $(M+2H-SO_2NH_2)^+$]; MS. m/z (FAB-) 484.1 [14, (M+NBA)'], 332.1 [100, $(M-H)^-$], 253.1 [50, $(M-SO_2NH_2)+$]; Acc. MS (FAB+) 334.0392, $C_{15}H_{12}NO_6S$ requires 334.0385. Found C, 54.0; H, 3.39; N, 4.21; $C_{15}H_{11}NO_6S$ requires C, 54.03; H, 3.33; N, 4.20%.

5-Hydroxy-7-methoxy-flavone-6-*O*-sulfamate (2): Upon sulfamoylation, 5,6-dihydroxy-7-methoxyflavone (500 mg, 1.760 mmol) gave a crude product (680 mg) which was fractionated by flash chromatography (chloroform/acetone, 8:1). The yellow residue that was obtained (463 mg, 72%) was further purified by recrystallization from acetone/hexane (1:2) to give **2** as yellow crystals (347 mg, 54%); mp 209-212°C; TLC (chloroform/acetone, 8:1, 4:1 and 2:1): R*f*s 0.13, 0.33, and 0.65 respectively; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3380 (NH_2), 3200 (OH), 1720 (C=O), 1380 ($-SO_2$), cm^{-1}; δ_H (270 MHz, DMSO-d_6) 3.95 (1H, s, OCH_3), 7.06 (1H, s, C-3-H or C-8-H), 7.11 (1H, s, C-8-H or C-3-H), 7.6 (3H, m, C-3'-H, C-4'-H and C-5'-H), 7.84 (2H, s, exchanged with D_2O, -OSO_2NH_2), 8.13 (2H, d, *J* = 7.0 Hz, C-2'-H and C-6'-H) and 13.06 (1H, s, C-5-OH); MS. m/z (FAB+) 364.0 [100, $(M+H)^+$], 284.1 [40, $(M+H-SO_2NH_2)^-$]; Acc. MS. (FAB+) 364.0511 $C_{16}H_{14}NO_7S$ requires 364.0491. Found C, 52.5; H, 3.68; N, 3.66; $C_{16}H_{13}NO_7S$ requires C, 52.89; H, 3.61; N, 3.85%.

5-Hydroxy-7-methoxyflavone-4'-*O*-sulfamate (3): Upon sulfamoylation, 4',5-dihydroxy-7-methoxyflavone (75 mg, 263.8 µmol) gave a crude product (112 mg) which was fractionated by flash chromatography (chloroform/acetone, 8:1). The yellow residue that was obtained (77 mg, 80%) was further purified by recrystallization from ethyl acetate/hexane (1:2) to give **3** as yellow crystals (55 mg, 57%); mp 189-192°C; TLC (chloroform/ acetone, 8:1 and 4:1): R*f*s 0.16 and 0.26 respectively; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3500 (NH_2), 3300 (OH), 1660 (C=O), 1380 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d_6) 3.94 (1H, s, C-7 -OCH_3), 6.36 (1H, d, *J* = 2.5 Hz, C-6-H or C-8-H), 6.73 (1H, d, *J* = 2.2 Hz, C-8-H or C-6-H), 6.84 (1H, s, C-3-H) 7.35 (2H, br s, exchanged with D_2O, -

SO_2NH_2), 7.54 (2H, d, *J* = 8.8 Hz, C-3'-H and C-5'-H or C-2'-H and C-6 '-H), 8.18 (2H, d, *J* = 8 . 8 Hz, C-2'-H and C-6 '-H or C-3'-H and C-5'-H) and 12.81 (1H, s, C-5-OH); MS. m/z (FAB+) 364.0 [100, (M+H)+], 285.1 [15, $(M-SO_2NH_2)^+$]; MS. m/z (FAB-) 361.9 [95, (M-H)-], 283.0 [100,$(M-SO_2NH_2)^-$]; Acc. MS. (FAB+) 364.0500 $C_{16}H_{14}NO_7S$ requires 364.0491.

5-Hydroxy-4',6,7-Trimethoxyflavone-3'-*O*-sulfamate (4): Upon sulfamoylation, 3',5-dihydroxy-4',6,7-trimethoxyflavone (75 mg, 217.8 µmol) gave a crude product (103 mg) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The yellow residue that was obtained (58 mg, 65%) was further purified by recrystallization from ethyl acetate/hexane (1:2) to give **4** as yellow crystals (32 mg, 36%); mp 197-199°C; TLC (chloroform/acetone, 4:1 and 2:1): *Rfs* 0.175 and 0.44 respectively; IR (KBr, cm^{-1}): ν = 3360 (NH2), 3260 (OH), 1620 (C=O), 1370 (SO_2) cm^{-1}; δ_H [270 MHz, DMSO-d_6/ $CDCl_3$, (ca 1:5)] 3.88 (3H, s, OCH_3), 3.95-3.986 (6 H, 2 x s, 2 x OCH_3), 6 . 6 6 (1H, s, C-3-H or C-8 -H), 6.71 (1H, s, C-8 -H or C-3-H), 7.16 (1H, d, *J* = 8. 8 Hz, C-5'-H or C-6 -H), 7.63 (2H, s, exchanged with D_2O, $-OSO_2NH_2$), 7.86 (1H, d, *J* = 8.8 Hz, C-5'-H or C-6 '-H), 7.97 (1H, d, *J* = 2.2 Hz, C-2'- H) and 12.74 (1H, s, C-5-OH); MS. m/z (FAB+) 424.1 [100, (M+H)+], 344.1 [10, $(M+H-SO_2NH_2)^+$]; MS. m/z (FAB-) 422.1 [100, $(M-H)^-$], 344.1 [20, $(M+H-SO_2NH_2)^-$]; Acc. MS. m/z (FAB+) 424.0714 $C_{18}H_{18}NO_9S$ requires 424.0702. Found C, 51.1; H, 3.38; N, 4.33; $C_{18}H_{17}NO_9S$ requires C, 51.06; H, 3.31; N, 4.05%.

4',5,6,7-Tetramethoxyflavone-3'-*O*-sulfamate (5): Upon sulfamoylation, 3'-hydroxy-4'-,5,6,7-tetramethoxyflavone (75 mg, 210 µmol) gave a crude product (96 mg) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The yellow residue that was obtained (59 mg, 6 6 %) was further purified by recrystallization from ethyl acetate/hexane (1:2) to give **5** as yellow crystals (35 mg, 39%); mp 138-141°C; TLC (chloroform/acetone, 8:1, 4:1 and 2:1): R*f*s 0.10, 0.27 and 0.59 respectively; IR (KBr, cm^{-1}): ν = 3420 (NH_2), 1640 (C=O), 1380 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d_6) 3.93 (12H, m, 4x OCH_3), 6.55 (1H, s, C-3-H or C-8 -H) 7.13 (1H, s, C-3-H or C-8 -H), 7.23 (2H, br s, exchanged with D_2O, $-OSO_2NH_2$) 7.24 (1H, d, *J*= 8.4 Hz, C-5'-H) and 7.93 (2H, m, C-2'-H and C-6 '-H); MS. m/z (FAB+) 437.9 [100, (M+H)+], 359.0 [10, (M+H-SO_2NH)+]; MS. m/z (FAB-) 436.0 [100, (M-H)+], 357.0 [10, $(M-H-SO_2NH_2)^-$]; Acc. MS (FAB+) 438.0866 $C_{19}H_{20}NO_9S$ requires 438.0859.

Flavone-3',4'-*O*,*O*-bis-sulfamate (6): Upopn sulfamoylation, 3',4'-dihydroxyflavone (450 mg, 1.717 mmol) gave a crude product (763 mg) which was fractionated by flash chromatography (ethyl acetate/acetone/hexane, 2:1:1). The residue that was obtained (376 mg, 53%) was further purified by recrystallization from acetone/hexane (1 :2) to give **6** as white crystals (173 mg, 25%); mp 170-172°C; TLC (ethyl acetate/acetone, 2:1 and ethyl acetate/acetone/hexane, 2:1:1): R*f*s 0.7 and 0.51 respectively; IR (KBr, cm^{-1}): ν = 3240 (NH_2), 1630 (C=O), 1390 (SO_2) cm^{-1}; δ_H [270 MHz, DMSO-d_6/ $CDCl_3$ (ca 1:3)] 6.91 (1H, s, C-3-H), 7.47-7.8 (5H, m, Ar-H), 7.97 (4H, br s, exchanged with D_2O, C-3 '- OSO_2NH_2 and C-4'-OSO_2NH_2), and 8.15 (2H, d, *J* = 7.7 Hz, Ar-H); MS m/z (FAB+) 413.1 [100, (M+H)+], 334.1 [20, (M+2H-SO_2NH_2)+], 254.1 [20, (M+H-2 x SO_2NH_2)+]; MS. m/z (FAB-) 411.0 [100, $(M-H)^-$], 332.1 [35, $(M-SO_2NH_2)^-$], 253.1 [40, (M+H-2 x $SO_2NH_2)^-$]; Acc . MS. 413.0120 $C_{15}H_{13}N_2O_8S_2$ requires 413.0113.

5-Hydroxyflavone-47-*O*,*O*-bis-sulfamate (7): Upon sulfamoylation, 4',5,7-trihydroxyflavone (72 mg, 258.4 µmol) gave a crude product (117 mg) which was fractionated by flash chromatography (chloroform/acetone, 2:1). The yellow residue that was obtained (70 mg, 63%) was further purified by recrystallization from acetone/chloroform (1:2) to give **7** as yellow crystals (45 mg, 32%); mp > 198°C (dec.); TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.083 and 0.385 respectively; IR (KBr, cm^{-1}): ν = 3380 (NH_2), 3260 (OH), 1650 (C=O), 1390 (SO_2) cm^{-1}; δ_H (270 MHz, DMSO-d_6) 6.76 (1H, s, C-3-H), 7.23 (2 H, d, *J* = 2.4 Hz, C-6 -H and C-8 -H), 7.5 (2H, d, *J* = 8.4 Hz, Ar-H of B-ring), 8.27 (2H, d, *J* = 9.1 Hz, Ar-H of B-ring), 8.38 (4H, br s, exchanged with D_2O, C-4'-OSO_2NH_2 and C-7-OSO_2NH_2) and 12.87 (1H, s, C-5-OH); MS. m/z (FAB+) 428.7 [80, (M+H)+], 349.8 [25, (M+2H-SO_2NH_2)+], 270.9 [25, (M+2H-2x SO_2NH_2)+]; MS. m/z (FAB-) 426.7 [80, $(M-H)^-$], 347.8 [80, $(M-SO_2NH_2)^-$], 268.9 [50, (M+H-2x$SO_2NH_2)^-$]; Acc. MS (FAB+) 429.0057 $C_{15}H_{13}N_2O_9S_2$ requires 429.0062.

5-Hydroxyflavone-3,7-*O*,*O*-bis-sulfamate (8): Upon sulfamoylation, 3,5,7-trihydroxyflavone (75 mg, 269.2 µmol) gave a crude product (0.123 g) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The yellow residue was obtained (58 mg, 54%) was further purified by recrystallization from ethyl acetate/hexane (1 :2) to give **8** as yellow crystals (31 mg, 28%); mp 171-173°C; TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.26 and 0.54 respectively; IR (KBr, cm^{-1}): ν = 3360 (NH_2), 3260 (OH), 1660 (C=O), 1390 (SO_2) c m^{-1}; δ_H (270 MHz, acetone-d_6) 6.81 (1H, d, 7 = 2.2 Hz, C-6 -H or C-8 -H), 7.21 (1H, d, *J* = 2.2 Hz, C-8 -H or C-6 -H), 7.31 (2H, br s, exchanged with D_2O , OSO_2NH_2), 7.54 (2H, br s, exchanged with D_2O, OSO_2NH_2), 7.64 (3H, m, Ar-H of B-ring), 8.14 (2H, m, Ar-H of B-ring) and 12.29 (1H, s, C-5-OH); M.S. m/z (FAB+) 428.9 [100, (M+H)+], 348.9 [50, (M+H-SO_2NH_2)+], 270.0 [25, (M+2H-2xSO_2NH_2)+]; MS. m/z (FAB-) 426.9 [85, (M-H)'], 347.9 [100, $(M-SO_2NH_2)^-$], 269.0 [80, (M+H-2x $SO_2NH_2)^-$]. Acc. MS: m/z 429.0086 $C_{15}H_{13}N_2O_9S_2$ requires 429.0063. Found C, 42.11; H, 3.06; N, 6.13; $C_{15}H_{12}N_2O_9S_2$ requires C, 42.06; H, 2.82; N, 6.54%.

5-Hydroxy-4'-methoxyflavone-3,7-*O*,*O*-bis-sulfamate (9): Upon sulfamoylation, 3,5,7-trihydroxy-4'-trimethoxyflavone (75 mg, 250 µmol) gave a crude product (0.116 g) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The yellow residue (54 mg, 47%) was further purified by recrystallization from acetone/hexane (1:2) to give **9** as yellow crystals (29 mg, 26%); mp > 192°C (dec.); TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.19 and 0.55 respectively; IR (KBr, cm^{-1}): ν = 3420 (NH_2), 1620 (C=O), 1360 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-ck) 3.94 (3H, s, OCHs), 6.80 (1H, s, Ar-H), 7.14 (3H, m, Ar-H of B-ring), 7.39 (2H, br s, exchanged with D_2O, $-OSO_2NH_2$), 7.50 (2H, s, exchanged with D_2O, $-OSO_2NH_2$), 8.2 (2H, d, 7= 6.7 Hz, Ar-H of B-ring) and 12.43 (1H, br s, C-5-OH); M.S. m/z (FAB+) 459.1 [100, (M+H)+], 379.1 [55, (M+H-SO_2NH_2)+], 300.1 [15, (M+2H-2x SO_2NH_2)']; MS. m/z (FAB-) 456.2 [20,(M-2H)'], 378.1 [100, (M-SO_2NH_2)], 299.1 [70, (M+H-H-2x $SO_2NH_2)^-$]; Acc. MS. 459.0174 $C_{16}H_{15}N_2O_{10}S_2$ requires 459.0168. Found C, 41.98; H, 3.06; N, 6.11; $C_{16}H_{14}N_2O_{10}S_2$ requires C, 41.92; H, 3.08; N, 6. 11%.

5-Hydroxy-3-',4',5'-Trimethoxyflavone-3,7-*O*,*O*-bis-sulfamate (10) and 5,7-dihydroxy-3',4',5'-trimethoxyflavone-3-*O*-sulfamate (11): Upon sulfamoylation, 3,5,7-trihydroxy-3',4',5'-trimethoxyflavone (200 mg, 558.4 µmol) gave a crude product (231 mg) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The yellow residue that was obtained (108 mg, 39%) was further purified by recrystallization from acetone/hexane (1 :2) to give **10** as yellow crystals (69 mg, 25%); mp 124-126 °C; TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.19 and 0.53 respectively; IR (KBr, cm^{-1}): ν = 3360 (NH_2), 3260 (OH), 1660 (C=O), 1430 (SO_2) cm^{-1}; δ_H [270 MHz, DMSO-d_6/ $CDCl_3$, (ca 1:3)] 3.91 (3H, s, OCH_3), 3.95 (6 H, s, 2xOCH_3), 6.83 (1H, d, *J* = 1.8 Hz, Ar-H of A-ring), 7.12 (1H, d, *J*= 2.2 Hz, Ar-H of A-ring), 7.39 (2H, s, C-2'-H and C-6'-H), 7.72 (2H, s, exchanged with D_2O, OSO_2NH_2), 8.06 (2H, s, exchanged with D_2O, OSO_2NH_2) and 12.2 (1H, s, C-5-OH); M.S. m/z (FAB+) 519.1 [100,

(M+H)+], 439.1 [60, (M+H-SO_2NH_2)+], 360.1 [10, (M+2H-2xS02NH2)"]; MS. m/z (FAB-) 517.1 [85,(M-H)'], 438.1 [100, (M-S02NH2)'], 359.1 [80, (M+H-2x SO_2NH_2)$^-$]; Acc. MS. 519.0387 $C_{18}H_{19}N_2O_{12}S_2$ requires 519.0379. The less polar fraction gave a yellow residue (53 mg, 22%) which was further purified by recrystallization from ethyl acetate/hexane (1:2) to give **11** as yellow crystals (26 mg, 11%); mp 189-192°C; TLC (chloroform/acetone, 8:1, 4:1 and 2:1): R*f*s 0.10, 0.32 and 0.62 respectively; IR (KBr, cm-1): $\bar{\nu}$ = 3500 (NH_2), 1660 (C=O), 1390 (SO_2) cm^{-1}; δ_H [270 MHz, DMSO-d6/ $CDCl_3$, 1:1) 3.84 (3H, s, OCH_3), 3.91 (6 H, s, 2x OCH_3), 6.27 (1H, s, Ar-H), 6.51 (1H, s, Ar-H), 7.33 (2H, s, C-2'-H and C-6 '-H), 7.84 (2H, br s, exchanged with D_2O, -OSO_2NH_2, 10.8 (1H, s, C-7-OH) and 12.43 (1H, s, C-5-OH); MS. m/z (FAB+) 440.1 [100, (M+H)+], 360.1 [80, (M+H-SO_2NH_2)+]; MS. m/z (FAB-) 438.1 [100, (M-H)-], 359.1 [80, (M-SO_2NH_2)-]; Acc. MS. 440.0633 $C_{18}H_{18}NO_{10}S$ requires 440.0651.

3',4',5'-Trimethoxyflavone-3,7-*O*,*O*-bis-sulfamate (12) and 7-hydroxy-3',4',5'-trimethoxyflavone-3-*O*-sulfamate (13): Upon sulfamoylation, 3,7-dihydroxy-3',4',5'-trimethoxyflavone (200 mg, 563.4 µmol) gave a crude product (285 mg) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The yellow residue that obtained (212 mg, 75%) was further purified by recrystallization from acetone/hexane (1 :2) to give **12** as light yellow crystals (123 mg, 44%); mp 182-184 °C; TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.14 and 0.21 respectively; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3480 (NH_2), 1610 (C=O), 1420 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d6) 3.85 (3H, s, OCH_3), 3.94 (6 H, s, 2xOCH_3), 7.48 (3H, m, C-6 -H, C-2'-H and C-6 '-H), 7.69 (4H, br s, exchanged with D_2O , 2x-OSO_2NH_2), 7.79 (1H, s, C-8 -H) and 8.27 (1H, d, *J* = 8.3 Hz, C-5-H); MS. m/z (FAB+) 502.9 [100, (M+H)+], 423.0 [60, (M+H- SO_2NH_2)+], 344.1[10, (M+2H-2x SO_2NH_2)+]; MS. m/z (FAB-) 501.0 [100, (M-H)'], 422.0 [80, (M-SO_2NH_2)], 343.0 [75, (M+H-2x SO_2NH_2)-]; Acc. MS 503.0444 $C_{18}H_{19}N_2O_{11}S_2$ requires 503.0430.

The less polar fraction gave **13** as light yellow residue (12 mg); m.p 159-162 °C; TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.23 and 0 .5 0 respectively; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3500 (NH_2), 3250 (OH), 1620 (C=O), 1420 (SO_2) cm^{-1}; MS m/z (FAB+) 424.0 [100, (M+H)+], 344.0 [70, (M+H- SO_2NH_2)+]; MS m/z (FAB-) 422.0 [100, (M-H)'], 343.0 [70, (M- SO_2NH_2)']; A cc MS m/z (FAB+) 424.0702 $C_{18}H_{18}NO_9S$ requires 424.0703.

5,7,-Dihydroxyflavone-6-*O*-sulfamate (14): Upon sulfamolyation, 5,6,7-trihydroxyflavone (400 mg, 1.480 mmol) gave a crude product (495 mg) which was fractionated by flash chromatography (chloroform/acetone gradient). The yellow residue was obtained (350 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **14** as yellow crystals (273 mg, 53%); mp 196-198 °C; TLC (chloroform/acetone, 8:1,4:1 and 2:1): R*f*s 0.15, 0.26 and 0.34 respectively; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3500-2500 (NH_2 and OH), 1670 (C=O), 1380 (SO_2) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 6.69 (1H, s, C-3-H), 7.04 (1H, s, C-8 -H), 7.63 (3H, m, C-3'-H, C-4'-H, and C-5'-H), 7.76 (2H, br s, exchanged with D_2O, -SO_2NH_2) 10.03 (1H, br s, C-7-OH) and 13.3 (1H, br s, C-5-OH); MS. m/z (FAB+) 349.9 [100, (M)+], 269.9 [50, (M-SO_2NH_2)+]; M.S. m/z (FAB-) 501 [10, (M-H-NBA)], 348.[100, (M-H)], 269.0 [50, (M- SO_2NH_2)$^+$]; Acc. MS. (FAB+) 350.03219 $C_{15}H_{12}NO_7S$ requires 350.03344. Found C, 51.9; H, 3.21; N, 3.86; $C_{15}H_{11}NO_7S$ requires C, 51.58; H, 3.17; N, 4.01%.

Flavone-4'-6-*O*,*O*-bis-sulfamate (15): Upon sulfamoylation, 4'-6-dihydroxyflavone (500 mg, 1.967 mmol), gave a crude product (730 mg) which was fractionated by flash chromatography (chloroform/acetone gradient). The yellow residue that was obtained (510 mg) was further purified by recrystallization from acetone/hexane (1:2) gave **15** as yellow crystals (365 mg, 45%); mp > 162°C (dec); TLC (chloroform/acetone, 8:1): R*f* 0.2; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3300 (-NH_2), 1630 (C=O), 1370 (-SO_2N-) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 7.12 (1H, s, C-3-H), 7.5 (2H, dd, $J_{C\text{-}2'\text{-}H,\ c\ \text{-}3''\ \text{-}H}$ = 8 . 8 Hz, C-3'-H and C-5'-H), 7.72 (1H, dd, $J_{C\text{-}5\text{-}H,\ C\text{-}7\text{-}H}$ = 2.7 Hz and $J_{C\text{-}8\text{-}H,\ C\text{-}7\text{-}H}$ = 9.2 Hz, C-7-H), 7.93 (2H, m, C-5-H and C-8 - H), 8.18 (4H, 2 br s, exchanged with D_2O, 2x-SO_2NH_2), and 8.25 (2H, d, *J* = 8.5 Hz, C-2'-H and C-6 '-H); MS. m/z (FAB+) 412.9 [100, (M+H)+], 329.9 [25, (M-H-SO_2NH_2)], 305.9 (20), 305.9 (15), 287.9 (20), 271.9 (25), 257.0 (20), 243.0 (30); MS. m/z (FAB-) 410.9 [100, (M-H)$^-$], 331.9 [85, (M-H-SO_2NH_2)], 292.0 (25), 276.0 (25), 258.0 (30), 215.0 (30); Acc. mass (FAB+) 413.0104 requires $C_{15}H_{13}N_2O_8S_2$ 413.0113. Found C, 43.17; H, 2.85; N, 6.53 requires $C_{15}H_{12}N_2O_8S_2$ C, 43.69; H, 2.93; N, 6.79%.

Flavone-4'-*O*-sulfamate (16): Upon sulfamolyation, 4'-hydroxyflavone (500 mg, 2.09 mmol) gave a crude product (640 mg) which was fractionated by flash chromatography (chloroform/acetone gradient). The pale white residue obtained (593 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **16** as white crystals (445 mg, 67%); mp 218-220°C; TLC (chloroform/acetone, 8:1): R*f* 0.2; IR (KBr, cm^{-1}): $\bar{\nu}$ = 3360 (NH_2), 1620 (C=O), 1390 (SO_2) cm^{-1}; δ_H (270 MHz, DMSO-d_6) 7.1 (1H, s, C-3-H), 7.5 (3H, m, C-8-H, C-3'-H, and C-5'-H), 7.83 (2H, m, C-6 -H and C-7-H), 8.08 (1H, dd, $J_{C\text{-}7\text{-}H,\ C\text{-}5\text{-}H}$ = 2 .1 Hz and $J_{C\text{-}6\text{-}H,\ C\text{-}5\text{-}H}$ = 7.7 Hz, C-5-H) and 8.25 (4H, m, 2H exchanged with D_2O, -SO_2NH_2 and C-2'-H and C-6'-H); MS. m/z (FAB+) 317.9 [100, (M)+], 238.9 [15, (M+H-SO_2NH_2)+], 173.0 (10); M.S. m/z (FAB-) 470.0 [25, (M-NBA)-], 316.0 [100, (M-H)$^-$], 237.0 [55, (M-SO_2NH_2)$^-$]; Acc. MS. (FAB+) 318.0445 $C_{15}H_{12}NO_5S$ requires 318.0436. Found C, 56.9; H, 3.4; N, 4.23; $C_{15}H_{11}NO_5S$ requires C, 56.78; H, 3.49; N, 4.41%.

Flavone-7-methane-sulfonate (17): To a stirred solution of 7-hydroxyflavone (100mg, 42 µmmol) in anhydrous pyridine (3 ml) at 0°C under N_2, methane sulfonyl chloride (0.2 ml, 2.584 mmol) was added dropwise. Subsequently, the reaction mixture was allowed to warm to room temperature, and stirring was continued overnight. The reaction mixture was then poured into an ice-water (20 ml) and extracted with ethyl acetate (5x 20 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. Final traces of pyridine were removed by repeating co-evaporation with toluene. The crude product was precipitated by methanol to give a yellow solid (110 mg), which recrystallized from methanol to give compound **17** as pale yellow crystals (90 mg, 6 8 %); mp 169-170°C (lit. 160°C) [37]; TLC (chloroform/acetone, 8:1): R*f* 0.61;

IR (KBr, cm^{-1}): $\bar{\nu}$ = 1630 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.26 (3H, s, -OSO_2CH_3), 6.85 (1H, s, C-3-H), 7.33 (1H, dd, $J_{C\text{-}8\text{-}H,\ C\text{-}6\text{-}H}$ = 2.4 Hz and $J_{C\text{-}5\text{-}H,\ C\text{-}6\text{-}H}$ = 8.8 Hz, C-6-H), 7.55 (3H, m, C-3'-H, C-4'-H, and C-5'-H), 7.6 (1H, d, $J_{C\text{-}6\text{-}H,\ C\ \text{-}8\text{-}H}$ = 2.1 Hz, C-8 -H), 7.95 (2H, dd, $J_{C\text{-}3'\text{-}H,\ C\ \text{-}2'\text{-}H}$ = 7.8 Hz and $J_{C\text{-}4'\text{-}H,\ C\ \text{-}2'\text{-}H}$ = 2.8 Hz, C-2'-H and C-6'-H) and 8.3 (1H, d, $J_{C\text{-}6\text{-}H,\ C\text{-}5\text{-}H}$ = 8.8 Hz, C-5-H); M.S. m/z (FAB+) 317.1 [90, (M+H)$^+$], 281.1 (15), 147 (50), 72.9 (100); Acc. MS. (FAB+) 317.0514 $C_{16}H_{12}O_5S$ requires 317.0484.

β-Naphthoflavone-4'-*O*-sulfamate (18): Upon sulfamoylation, 4'-hydroxy β-naphthoflavone (200 mg, 693.7 µmol) gave a crude product (220 mg) which was fractionated by flash chromatography (chloroform/acetone gradient). The pale white residue that was obtained (186 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **18** as white crystals (150 mg, 59%); mp

245-247°C; TLC (chloroform/acetone, 8:1): R*f* 0.25; IR (KBr, cm^{-1}): -

v = 3300 (NH_2), 1630 (C=O), 1380 (SO_2) cm^{-1}; δ_H (270 MHz, DMSO-d_6) 7.26 (1H, s, C-3-H), 7.5 (2 H, d ,*J* = 8 . 8 Hz, C-3'-H and C-5'-H), 7.7 (1H, t, *J* = 7.5 Hz, C-7-H or C-8 -H) 7.81 (1H, t, *J* = 7.3 Hz, C-7-H or C-8 -H), 7.92 (1H, d , *J*= 8.9 Hz, C10-H or C-11-H), 8.12 (1H, d, *J* = 7.9 Hz, C -11-H or C-10-H), 8.23 (2H, br s, exchanged with D_2O, $-SO_2NH_2$), 8.3 (2H, d, *J* = 8 . 6 Hz, C-2'-H and C-6 '-H), 8.4 (1H, d, *J* = 9.2 Hz, C-9-H) and 9.96 (1H, d, *J* = 8 . 6 Hz, C-9-H); MS. m/z (FAB+) 367.9 [100, $(M)^+$], 349.0 (30), 330.0 (70), 319.0 (25), 305.0 (40) 287.0 [55, $(M\text{-}SO_2NH_2)^+$], 272.0 (70), 257.1 (80), 243.0 (75); MS. m/z (FAB-) 366.0 [100, (M-H)], 349.0 (55), 328.0 (50), 317.0 (45), 292.1 (45), 276.0 (75), 258.0 (70), 243.0 (25); Acc. MS. m/z 368.0584 $C_{19}H_{14}NO_5S$ requires 368.0592. Found C, 61.9; H, 3.45; N, 3.63; $C_{19}H_{13}NO_5S$ requires C, 62.12; H, 3.57; N, 3.81%.

5-Hydroxy-4'-methoxyisoflavone-7-*O*-sulfamate (19): Upon sulfamoylation, 5,7-dihydroxy-4'-methoxyisoflavone (800 mg, 2.817 mmol) gave crude product (650 mg) which was fractionated by flash chromatography (chloroform/acetone, 8:1). The yellow residue that was obtained (266 mg, 26%) was further purified by recrystallization from ethyl acetate/hexane (1:1) to give **19** as yellow crystals (211 mg); mp 184-188°C; TLC (chloroform/acetone, 8:1 and 4:1): Rf 0.22 and 0.59 respectively; IR (KBr, cm-1): -

v = 3300 (NH_2), 1660 (C=O), 1400 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d_6) 3.86 (3H, s, OCH_3), 6.75 (1H, d, *J* = 2.2 Hz, C-6-H or C-8-H), 7.04 (3H, m, C-6-H or C-8-H, and Ar-H of B-ring), 7.49 (2H, br s, exchanged with D_2O, OSO_2NH_2), 7.58 (2H, d, *J* = 7.0 Hz, Ar-H of B-ring), 8.41 (1H, s, C-2-H), 13.05 (1H, br s, C-5-OH); MS. m/z (FAB+) 364.0 [100, $(M+H)^+$], 284.1 [12, $(M+H\text{-}SO_2NH_2)^+$]; M.S. m/z (FAB-) 515.1 [12, $(M\text{-}H+NBA)^-$], 362.1 [100, $(M\text{-}H)^-$], 283.1 [70, $(M\text{-}SO_2NH_2)^-$]; Acc. MS. (FAB+) 364.0494 $C_{16}H_{14}NO_7S$ requires 364.0491. Found: C, 52.8; H, 3.65; N, 3.81; $C_{16}H_{13}NO_7S$ requires C, 52.89; H, 3.61; N, 3.85%.

5-Hydroxyisoflavone-4',7-*O*,*O*-bis-sulfamate (20) and 5,7-dihydroxyisoflavone-4'-*O*-sulfamate (21): Upon sulfamoylation, 4',5,7-trihydroxyisoflavone (500 mg, 1.85 mmol) gave a crude product (650 mg) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The light yellow residue that was obtained (329 mg, 42%) was further purified by recrystallization from ethylacetate/hexane (1:2) to give 2 0 as beige crystals (197 mg, 25%); mp > 198 °C (dec.); TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.14 and 0.24 respectively; IR (KBr, cm^{-1}): -

v = 3460 (NH_2), 1650 (C=O), 1400 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d_6) 6.78 (1H, d, *J* =2.2 Hz, C-6 -H or C-8 -H), 7.03 (1H, d, *J* = 2.2 Hz, C-8 -H or C-6 -H), 7.4 (4H, br s, exchanged with D_2O, $2xOSO_2NH_2$), 7.43 (2H, d, *J* = 8.4 Hz, Ar-H of B-ring), 7.72 (2H, d, J = 8.4 Hz, Ar-H of B-ring), 8.51 (1H, s, C-2-H) and 12.93 (1H, s, C-5-OH); MS. m/z (FAB+) 428.9 [100, $(M+H)^+$], 350.0 [20, $(M+2H\text{-}SO_2NH_2)^+$], 272.1 (30); MS. m/z (FAB-) 426.9 [100, $(M\text{-}H)^-$], 347.9 [95, $(M\text{-}SO_2NH_2)^-$], 269.0 [30, $(M+H\text{-}2xSO_2NH_2)^-$]; Acc. MS m/z (FAB+) 429.0083 $C_{15}H_{13}N_2O_9S_2$ requires 429.0063. Found C, 42.0; H, 2.91; N, 6.45; $C_{15}H_{12}N_2O_9S_2$ requires C, 42.06; H, 2.82; N, 6.54%.

The less polar fraction gave **21** as light yellow residue (112 mg, 17%) which was further purified by recrystallization from ethyl acetate/hexane (1:3) to give **21** as pale white crystals (6 8 mg, 10%); mp 189-192 °C; TLC (chloroform/acetone, 4:1 and 2:1) R*f*s 0.23 and 0.33 respectively; IR (KBr, cm^{-1}): -

v = 3500-3300 (NH_2), 3200 (OH), 1680 (C=O), 1610, 1400 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d_6) 6.32 (1H, d, *J*= 2.2Hz, C-6 -H or C-8-H), 6.46 (1H, d, .*J* = 2.2 Hz, C-8 -H or C-6 -H), 7.32 (2H, br s, exchanged with D_2O, $-SO_2NH_2$), 7.42 (2H, d, *J* = 8.4 Hz, Ar-H of B-ring), 7.69 (2H, d, *J* = 8.4 Hz, Ar-H of B-ring) 8.31 (1H, s, C-2-H), 9.53 (1H, s, C-7-OH) and 12.9 (1H, s, C-5-OH); MS. m/z (FAB+) 350.0 [100, $(M+H)^+$], 271.1 [15, $(M+2H\text{-}SO_2NH_2)^+$]; MS. m/z (FAB-) 347.9 [100, $(M\text{-}H)^-$], 269.0 [20, $(M\text{-}SO_2NH_2)^-$]; Acc. MS (FAB+) 350.0347 $C_{15}H_{12}NO_7S$ requires 350.0335. Found C, 51.0; H, 3.16; N, 3.90; $C_{15}H_{11}NO_7S$ requires C, 51.58; H, 3.17; N, 4.01%.

Isoflavone-4',7-*O*,*O*-bis-sulfamate (22): Upon sulfamoylation, 4',7-dihydroxyisoflavone (450 mg, 1.717 mmol) gave a crude product (769 mg) which was fractionated by flash chromatography (chloroform/acetone, 4:1). The pale white residue (553 mg, 70%) was further purified by recrystallization from acetone/hexane (1:2) to give **22** as white crystals (327 mg, 41%); mp > 195°C (dec.); TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.21 and 0.40 respectively; IR (KBr, cm^{-1}): -

v = 3400 (NH_2), 1640 (C=O), 1360 (SO_2) cm^{-1}; δ_H (270 MHz, DMSO-d_6) 7.37 (2H, d, *J* = 8 . 8 Hz, Ar-H of B-ring), 7.42 (1H, dd, $J_{C\text{-}8\text{-}H,\ C\text{-}6\text{-}H}$ = 2.2 Hz, $J_{C\text{-}5\text{-}H,\ C\text{-}6\text{-}H}$ = 8.8 Hz, C-6 -H), 7.7 (2H, d, *J* = 8.8 Hz, Ar-H of B-ring), 8.09 (2H, br s, exchanged with D_2O, OSO_2NH_2), 8.24 (1H, d, *J* = 8.8 Hz, C-5-H), 8.36 (2H, br s, exchanged with D*2*O, OSO_2NH_2 and 8.63 (1H, s, C-2-H); MS. m/z (FAB+) 412.9 [100, $(M+H)^+$], 334.0 [25, $(M+2H\text{-}SO_2NH_2)$+], 255.1 (20); MS m/z (FAB-) 410.9 [100, $(M.H.)^-$], 332.0 [70, $(M\text{-}SO_2NH_2)^-$], 253.0 [30, $(M+H\text{-}2xSO_2NH_2)^-$]; Acc. MS. m/z (FAB+) 413.0119 $C_{15}H_{13}N_2O_8S_2$ requires 413.0113. Found C, 44.0; H, 2.94; N, 6.62; $C_{15}H_{12}N_2O_8S_2$ requires C, 43.69; H, 2.93; N, 6.79%.

(±) 5,7-Dihydroxyflavanone-4'-*O*-sulfamate (23): Upon g sulfamoylation, 4', 5,7-trihydroxy flavanone (1.0 g, 3.675 mmol) gave crude product (965 mg), which was fractionated by flash chromatography (chloroform/acetone gradient). The pale yellow oil obtained (345 mg, 27%), solidified on standing, was subsequently further purified by recrystallization from ethyl acetate/hexane (1:1) gave 23 as white crystals (259 mg, 20%); mp 211-213°C; TLC (chloroform/acetone, 4:1) R/0.21; IR (KBr, cm^{-1}): -

v = 3420 (NH_2), 3260 (OH), 1640 (C=O), 1380 (SO_2N) cm^{-1}; δ_H (400 MHz, acetone-d_6) 2.84 (1H, dd, J_{AB} = 17.4 Hz and $J_{ax,eq}$= 3.1 Hz, C -3-H_B), 3.19 (1H, dd, J_{BA}= 16.9 Hz and $J_{ax,ax}$ = 12.8 Hz, C-3-H_A), 5.62 (1H, dd, $J_{ax.eq}$ = 3.1 Hz and $J_{ax,ax}$= 12.8 Hz, C-2-H), 5.9 (1H, d, *J* = 2.0 Hz, C-6-H or C-8-H), 6.01 (1H, d, *J* = 2.0 Hz, C-6-H or C-8-H), 7.20 (2H, br s, exchanged with D_2O, $-OSO_2NH_2$), 7.40 (2H, d, *J* = 8.7 Hz, Ar-H of B-ring), 7.66 (2H, d, *J* = 8.7 Hz, Ar-H of B-ring), 9.65 (1H, br s, C-7-OH) and 12.15 (1H, s, C-5-OH); MS. m/z (FAB+) 352.0 [100, $(M+H)^+$], 272.1 [14, $(M+H\text{-}SO_2NH_2)$+]; MS. m/z (FAB-) 504.1 [20, $(M+NBA)^-$], 350.1 [100, $(M\text{-}H)^-$], 271.1 [45, $(M\text{-}SO_2NH_2)^-$]; Acc. MS. (FAB+) 352.0496 $C_{15}H_{14}NO_7S$ requires 352.0491. Found C, 51.1; H, 3.68; N, 3.98; $C_{15}H_{13}NO_7S$ requires C, 51.28; H, 3.73; N, 3.99%.

(±) 5-Hydroxy-4'-methoxyflavanone-3',7-*O*,*O*-bis-sulfamate (24): Upon sulfamoylation, 4'-methoxy-3',5,7-trihydroxyflavanone (1.0 g, 3.308 mmol) gave a crude product (1.672 g)

which was fractionated by flash chromatography (chloroform/acetone, 2:1). The yellow residue that was obtained (715 mg, 47%) was further purified by recrystallization from ethyl acetate/hexane (1:2) to give **24** as yellow crystals (513 mg); mp 186-188°C; TLC (chloroform/acetone, 4:1 and 2:1): R*f* 0.13 and 0.35 respectively; IR (KBr, cm^{-1}): -

ν = 3380 (NH_2), 3280 (OH), 1640 (C=O), 1390 (SO_2) cm^{-1}; δ_H (270 MHz, acetone-d_6) 2.92 (1H, m, C-3-H_A), 3.36 (1H, m, C-3-H_B), 3.91 (3H, s, OCH_3), 5.68 (1H, dd, $J_{ax,ax}$ = 12.8 Hz, and = 3.2 Hz, C-2-H), 6.47 (2H, d, $J_{C\text{-}6\text{-}H,\ C\text{-}8\text{-}H}$ = 2.8 Hz, C-6-H and C-8-H), 7.07 (2H, br s, exchanged with D_2O, -OSO_2NH_2), 7.24 (1H, d, $J_{C\text{-}5'\text{-}H,\ C\text{-}6'\text{-}H}$ = 8.4 Hz, C-5'-H), 7.50 (2H, br s, exchanged with D_2O, -OSO_2NH_2), 7.55 (1H, dd, $J_{C\text{-}5'\text{-}H.\ C\text{-}6'\text{-}H}$ = 8.4 Hz, $J_{C\text{-}2'\text{-}H.\ C\text{-}6'\text{-}H}$ = 2.2 Hz, C-6 '-H), 7.75 (1H, d, $J_{C\text{-}2'\text{-}H,\ C\text{-}6''\text{-}H}$ = 2.2 Hz, C-2'-H) and 12.02 (1H, s, C-5-OH); MS. m/z (FAB+) 460.9 [100, $(M+H)^+$], 381.9 [20, $(M+2H\text{-}SO_2NH_2)^+$], 303.0 (25); MS. m/z (FAB-) 458.9 [100, $(M\text{-}H)^-$], 379.9 [20, $(M\text{-}SO_2NH_2)^-$], 301.0 [20, $(M+H\text{-}2xSO_2NH_2)^-$]; Acc. M.S. (FAB+) 461.0337 $C_{16}H_{17}N_2O_{10}S2$ requires 416.0325.

(±) 4',7-Dihydroxy-isoflavane (Equol) (25): A suspension of Pd-C (10%, 2.2 g) was stirred under air in glacial acetic acid (15 ml) for three days before adding to a solution of 4', 7-dihydroxy isoflavone (500 mg, 1.906 mmol) in diglyme (60 ml). After 30 minutes of catalytic hydrogenation at balloon pressure, the reaction mixture was filtered, and the residue was washed with diglyme and hot acetic acid. The combined filtrates were evaporated and the crude obtained was dissolved in ethyl acetate, washed with 5% aqueous sodium bicarbonate (100 ml), water, dried ($MgSO_4$), filtered and evaporated to give a pale white crude (385 mg) which was recrystallized from aqueous ethanol (90%) to give **25** as white crystals (361 mg, 81%); mp 156-158 °C (lit. 158 °C) [37]; TLC (chloroform/acetone, 8:1 and 4:1): R*f*s 0.53 and 0.36 respectively; IR (KBr, cm^{-1}): -

ν = 3380-2720 cm'1; δ_H (400 MHz, acetone-d_6, cosy) 2.89 (2H, m, C-4 -H_2), 3.1 (1H, m, C-3-H), 3.94 (1H, t, J = 10.4 Hz, C-2-H_A), 4.18 (1H, m, C-2-H_E), 6.29 (1H, d, $J_{C\text{-}6\text{-}H,\ C\text{-}8\text{-}H}$ = 2.4 Hz, C-8-H), 6.37 (1H, dd, $J_{C\text{-}7\text{-}H,\ C\text{-}6\text{-}H}$ = 8.2 Hz, $J_{C\text{-}8\text{-}H,\ C\text{-}6\text{-}H}$ = 2.4 Hz, C-6-H), 6.82 (2H, dd, $J_{C\text{-}2'\text{-}H,\ C\text{-}3'\text{-}H}$= 8.6 Hz, $J_{C\text{-}5'\text{-}H,\ C\text{-}3'\text{-}H}$= 2.7 Hz,C-3'-H and C-5'-H or C-2'-H and C-6'-H), 6.89 (1H, d, $J_{C\text{-}6\text{-}H,\ C\text{-}5\text{-}H}$= 8.2 Hz, C-5-H), 7.16 (2H, dd, $J_{C\text{-}5'\text{-}H,\ C\text{-}2'\text{-}H}$= 8.2 Hz, $J_{C\text{-}6'\text{-}H.\ C\text{-}2'\text{-}H}$= 2.7 Hz, C-2'-H and C6'-H or C-3'-H and C-5'-H), 8.15 (1H, br s, C-7-OH or C-4'-OH) and 828 (1H, br s, C4'-OH or C-7-OH); δ_H (400 MHz, acetone-d_6, cosy) 32.64 (t, C-4), 38.77 (d, C-3), 71.56 (t, C-2), 103.64 (d, C-8), 108.83 (d, C-6), 114.04 (s), 116.28 (d, C-3' and C-5'), 129.24 (d, C-2' and C-6'), 133.39 (s, C-1'), 155.96 (s), 157.19 (d, C-7 or C-4') and 157.55 (d, C-4' or C-7); MS. m/z (FAB+) 242.1 [100, $(M)^+$], 213.1 (5), 185.1 (5), 177.1 (10), 167.1 (25), 152.1 (30), 135.0 (40), 123.0 (45), 105.0 (35), 95.0 (30), 78.9 (30), 68.9 (35), 56.9 (55); MS. m/z (FAB-) 393.2 [55, (M-H+NBA)'], 241.2 [100, (M-H)-], 227.1 (10), 215.1 (15), 198.1 (30), 181.1 (20), 162.1 (10), 139.1 (40), 124.1 (20), 106.0 (25), 92.0 (25) and 62.0 (10); Acc. MS. (FAB+) 243.1001 $C_{15}H_{15}O_3$ requires 243.1021. Found C, 74.3; H, 5.77; $C_{15}H_{14}O_3$ requires C, 74.36; H, 5.82%.

(+) Isoflavane-4',7-*O*,*O*-bis-sulfamate (26): Upon sulfamoylation, 25 (150 mg, 619.8 µmol) gave the crude product (254 mg), which was fractionated by flash chromatography (chloroform/acetone gradient). The brown residue that was obtained (96 mg, 38%) was further purified by recrystallization from acetone/hexane (1:2) to give **26** as light brown crystals (61 mg, 24%); mp > 120°C (dec.); TLC (chloroform/acetone, 4:1 and 2:1): R*f*s 0.22 and 0.34 respectively; IR (KBr, cm^{-1}): -

ν = 3160 (NH_2), 1380 (SO_2) cm^{-1}; δ_H (400 MHz, acetone-d_6) 3.1 (2H, m, C-4-H_2), 3.13 (1H, m, C-3-H), 4.12 (1H, t, J = 10.4 Hz, C-2-H_A), 4.35 (1H, dd, J_1 = 3.1 Hz and J_2= 10.7 Hz C-2 -H_{eq}), 6.78 (1H, d, $J_{C\text{-}6\text{-}H,\ C\text{-}8\text{-}H}$= 2.4 Hz, C-8 -H), 6.84 (1H, dd, $J_{C\text{-}7\text{-}H,\ C\text{-}6\text{-}H}$= 8.4 Hz, $J_{C\text{-}8\text{-}h,\ C\text{-}6\text{-}H}$= 2.4 Hz, C-6 -H), 7.13 (4H, br s, exchanged with D_2O, $2XOSO_2NH_2$), 7.18 (1H, d, $J_{C\text{-}6\text{-}H\ ,\ C\text{-}5\text{-}H}$= 8.2 Hz, C-5-H), 7.32 (2H, m, Ar-H of B-ring), 7.44 (2H, m, Ar-H of B-ring); MS. m/z (FAB+) 400.0 [100, $(M)^+$], 321.1 [40, $(M+H\text{-}SO_2NH_2)^+$], 243.2 (25); MS. m/z (FAB-) 553.0 [15, $(M+NBA)^-$], 399.0 [100, $(M\text{-}H)^-$], 96.0 (10), 77.9 (20); Acc. MS. (FAB+) 400.0405 $C_{15}H_{16}N_2O_7S_2$ requires 400.0399. Found: C, 44.6; H, 4.0; N, 6.54; $C_{15}H_{16}N_2O_7S_2$ requires C, 44.99; H, 4.03; N, 7.0%.

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