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# PLANT POLYPHENOLS: BIOLOGICALLY ACTIVE COMPOUNDS OR NON-SELECTIVE BINDERS TO PROTEIN?

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**Key Word Index**—radioligand receptor binding assays; polyphenols; tannins; proanthocyanidins; gallate/hexahydroxyldiphenic acid esters of glucose.

**Abstract**—Twenty phenolic compounds, representatives of proanthocyanidins and gallic acid/hexahydroxyldiphenic acid esters of glucose, have been assessed for their ability to inhibit binding of specific radioligands to 16 receptors. The receptors utilized were α1-, α2- and β-adrenoceptors, adenosine 1, dopamine 1 and 2, muscarinic, $Ca^{2+}$ channel, sulphonylureas, 5HT1, 5HT1A, 5HT2, histamine 1, benzodiazepine, opiate and $Na^+/K^+$ATPase. These phenolic compounds failed to inhibit ligands binding to 10 of the receptors under the test conditions. The most susceptible receptors to phenolic binding were β-adrenergic, 5HT1 and opiate. Some of the compounds tested showed selectivity for a single or for two receptors. The inhibition of binding of radioligands to receptors by the phenolic compounds cannot be explained solely in terms of phenolic–protein binding. The results indicate that the removal of tannins from plant extracts prior to screening for receptor activities may result in missing biologically active compounds with specificity of action. Copyright © 1997 Elsevier Science Ltd

## INTRODUCTION

Tannins (polyphenols) are well known for their ability to react with proteins as they precipitate albumin, gelatin and other proteins from aqueous solutions and are used to convert animal hide into leather by cross linking collagen molecules [1]. Many herbs which are used for medicinal purposes contain astringent (i.e. binding properties) tannins which are regarded as their active principles. Such herbs are recommended for a wide range of treatments including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, stomach disorders, nervous and hormonal problems, and inhibition of active oxygen, such as inhibition of lipid peroxidation and lipoxygenase, xanthine oxidase and monoamine oxidase [1–3]. Commonly, such herbs are recommended as diuretics, antidiarrhoeals and haemostatics. Examples of herbs containing tannins include *Geranium thunbergii* aerial parts and *Paeonia lactiflora* roots which are widely used in eastern medicine and the leaves of *Crataegus* species and *Rubus idaeus* which are used in western herbal medicine. Of current interest there is a 'green tea' which is highly regarded in eastern cultures, allegedly for its antioxidant, antitumour, radioprotective and antimutagenic activities of tannins [4, 5]. In a recent report of a cross sectional study of 1371 Japanese men of 40 years or older, it has been proposed that green tea acts protectively against cardiovascular diseases and disorders of the liver [6]. However, these natural products are not necessarily harmless and there are literature reports of oesophageal cancer being linked to ingestion of herbal teas containing tannins [7].

Vegetable tannins are water-soluble phenolic compounds having molecular weights between 500 and 3000 which have in addition to the usual phenolic properties the ability to precipitate proteins. The precipitation of gelatin is not entirely a reliable property to characterize tannins which must be sufficiently large to cross link collagen chains in skin so as to tan hide into leather. Phenolic compounds with $M_r$ below 500 are generally unable to cross link collagen whereas phenols with $M_r$ above 3000 are unable to penetrate collagen fibres. Nevertheless, it is the phenolic group which binds to protein and lower molecular weight phenols may bind without necessarily causing precipitation from aqueous solutions.

Tannins fall into two groups the proanthocyanidins (also known as condensed tannins) and polyesters of gallic acid or hexahydroxydiphenic acids (the hydrolysable tannins). The proanthocyanidins are typical of such plants as hawthorn and rhubarb whereas the

Table 1. Chemical structures and molecular weights of 20 phenolic compounds assessed for their ability to inhibit binding specific radioligands to 16 receptor preparations

| Compound (abbreviation) | $M_r$ | Chemical structure |
|---|---|---|
| Catechin (CA) | 290 | **1.** R1 = R2 = H |
| Gallocatechin (GC) | 306 | **2.** R1 = R2 = OH |
| Catechin 3-*O*-gallate (CG) | 442 | **3.** R1 = H; R2 = G |
| Epiafzelechin (EAF) | 274 | **4.** R1 = R2 = R3 = H |
| Epicatechin (EC) | 290 | **5.** R1 = R3 = H; R2 = OH |
| Epigallocatechin (EGC) | 306 | **6.** R1 = R2 = OH; R3 = H |
| Epigallocatechin 3-*O*-gallate (EGCG) | 458 | **7.** R1 = R2 = OH; R3 = G |
| Procyanidin B-2 (PB-2) | 578 | **8.** 3⋯⋯, 3′⋯⋯, 4-8— |
| Procyanidin B-3 (PB-3) | 578 | **9.** 3—, 3′—, 4-8⋯⋯ |
| Procyanidin B-4 (PB-4) | 578 | **10.** 3—, 3′⋯⋯, 4-8⋯⋯ |
| β-2,4-Di-*O*-galloyl-D-glucose (2DGG) | 484 | **11.** R1 = R3 = R5 = H, R2 = R4 = G |
| β-1,2,6-Tri-*O*-galloyl-D-glucose (2TGG) | 636 | **12.** R1 = R2 = R5 = G, R3 = R4 = H |
| β-1,3,6-Tri-*O*-galloyl-D-glucose (3TGG) | 636 | **13.** R1 = R3 = R5 = G, R2 = R4 = H |
| β-1,2,4,6-Tetra-*O*-galloyl-D-glucose (4TGG) | 788 | **14.** R1 = R2 = R4 = R5 = G, R3 = H |
| β-1,2,3,4,6-Penta-*O*-galloyl-D-glucose (PGG) | 940 | **15.** R1 = R2 = R3 = R4 = R5 = G |
| Tellimagrandin I (1HHDP) | 786 | **16.** R1 = H, R2 = R3 = G, R4 = R5 = G–G |
| Pedunculagin (2HHDP) | 784 | **17.** R1 = H, R2–R3 = R4–R5 = G–G |
| Davidiin (Dav) | 938 | **18.** R1–R5 = G–G, R2 = R3 = R4 = G |
| Geraniin (Ger) | 934 | **19.** |
| Rugosin-D (RD) | 1874 | **20.** |

galloyl and hexahydroxyldiphenoyl esters are found in rosehip, oak and geranium. The ability of phenols and polyphenols to bind with protein and to inhibit a small number of enzymes such as β-glucosidase is known but there is no detailed report of the effects which such compounds have at the receptor level.

## RESULTS AND DISCUSSION

Twenty phenolic natural products (Table 1) representing the two molecular types found in tannins, i.e. the proanthocyanidin flavan-3-ol monomers (**1**–**7**) and dimers (**8**–**10**), and the galloyl/hexahydroxyphenic acid esters of glucose (**11**–**20**), have been assessed for their ability to inhibit binding of specific radioligands to 16 different receptor preparations. Phenols (**1**–**20**) were tested at $10^{-5}$ M concentrations and the methodology used is described in the Experimental. The results are presented in Table 2.

Phenolic compounds bind to protein and tannins, and in particular are well known for their strong protein binding activities, thus it might well have been anticipated that there would be no significant differences in the abilities of compounds **1**–**20** to inhibit binding of radioligands to receptors. Surprisingly, all of these phenolic compounds at $10^{-5}$ M concentrations failed to significantly inhibit binding of specific radioligands (3+, Table 2) to 10 of the 16 receptors under the test conditions utilized. The inhibition of ligand binding to receptor was less than 60% for the following receptors, α1-adrenoceptor, 5HT1A, 5HT2, adenosine 1, benzodiazepine, $Ca^{2+}$ channel, sulphonylureas, muscarinic, histamine 1 and $Na^+/K^+$ ATPase. Indeed, for the majority of these 10 receptors, inhibition of ligand binding due to phenols **1**–**20** was generally less than 40% (+/±, Table 2) and frequently even negative. Those receptors which proved to be most susceptible to competitive binding by the phenolic compounds (**1**–**20**) were the β-adrenoceptor (5 compounds > 3+), 5HT1 (4 compounds > 3+) and opiate (5 compounds > 3+)

Under the test conditions, the phenols **1**–**20** were mainly inactive (3+) as competitors to binding of radioligands to the majority of the 16 receptors having activity against only one or two receptors (Table 2). Those compounds with activities of more than 60% inhibition of ligand binding ( > 3+) are summarized in Table 3. Of the 16 active compounds, 12 (**4**–**6**, **8**, **9**, **11**, **12**, **15**, **17**–**20**) were assessed as being active selectively against a single receptor including α2, β-adrenoceptor, 5HT1, opiate and dopamine 1 receptors; four compounds (**3**, **10**, **14**, **17**) were able to bind two receptors (Table 2). The remaining four compounds (**1**, **2**, **7**, **16**) were judged as being relatively inactive in that they failed to inhibit binding of specific radioligands to any of the 16 receptors (less than 60%, Table 2).

Polyphenol complexation to protein is mainly through hydrogen bond and hydrophobic interactions. In general, the extent of binding is related to molecular weight of polyphenols and this has been demonstrated for galloyl esters by several different methods including precipitation of haemoglobin from aqueous solution [1]. In order of increasing molecular weight the four tannins, β-1,2,6-tri-*O*-galloyl-D-glucose (**12**), β-1,2,3,6-tetra-*O*-galloyl-D-glucose, β-1,2,3,4,5-penta-*O*-galloyl-D-glucose (**15**) and rugosin (**20**) showed increasing ability to precipitate hae-

Table 2. Inhibition of radioligand binding to 16 receptors by phenolic compounds (**1–20**) at $10^{-5}$ M concentration*

| Receptor | Phenolic compounds | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** |
| α1-Adrenoceptor | – | ± | – | + | ± | ± | – | ± | + | 2+ | – | ± | – | – | – | + | – | – | + | ± |
| α2-Adrenoceptor | ± | ± | ± | ± | ± | ± | ± | ± | ± | 3+ | ± | + | ± | 3+ | 2+ | + | 2+ | 2+ | 2+ | 3+ |
| β-Adrenoceptor | 2+ | + | ± | 3+ | 3+ | ± | + | ± | + | + | + | 3+ | 3+ | + | ± | ± | 3+ | 2+ | ± | 2+ |
| Adenosine 1 | – | – | + | – | – | – | – | ± | – | – | – | – | – | – | – | – | – | – | – | – |
| Dopamine 1 | ± | – | 3+ | – | ± | ± | 2+ | ± | + | ± | + | + | – | ± | 2+ | ± | + | ± | 3+ | 2+ |
| Dopamine 2 | ± | – | + | ± | 2+ | 2+ | + | ± | + | 2+ | + | – | 2+ | 3+ | 2+ | + | ± | 2+ | – | – |
| Muscarinic | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | ± | – | – |
| $Ca^{2+}$ channel | ± | ± | ± | – | ± | ± | – | ± | ± | ± | ± | – | ± | – | ± | – | – | – | – | ± |
| Sulphonylureas | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | + | + | + | + | – | – | ± |
| 5HT1 | + | ± | 2+ | – | ± | 4+ | – | 4+ | 4+ | 4+ | 2+ | – | – | – | – | – | – | – | 2+ | – |
| 5HT1A | + | – | ± | + | ± | ± | ± | 2+ | ± | – | ± | ± | ± | ± | – | ± | ± | ± | – | ± |
| 5HT2 | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | + | – | – |
| Histamine 1 | ± | – | ± | – | ± | ± | ± | – | ± | 2+ | ± | ± | ± | ± | ± | ± | ± | ± | + | + |
| Benzodiazepine | – | – | – | – | – | ± | ± | ± | ± | ± | – | – | ± | – | – | – | – | – | ± | – |
| Opiate | ± | ± | 3+ | + | – | ± | 2+ | ± | + | ± | 3+ | 2+ | 3+ | 2+ | 3+ | 2+ | 2+ | 3+ | + | + |
| $Na^{-}/K^{+}ATP_{ase}$ | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

*Compounds were tested at $10^{-5}$ M in 1% aqueous MeOH, 50 μl aliquots tested; 4+: inhibition of 81–100%; 3+: inhibition of 61–80%; 2+: inhibition of 41–60%; +: inhibition of 21–40%; ±: inhibition of 1–20%; –: no inhibition. Inhibition % is based on means of two separate determinations in which each sample was triplicated.

moglobin from aqueous solution [1]. The results presented in Table 3 do not substantiate this molecular weight relationship at the receptor level, neither for the galloyl/hexahydroxydiphenic acid esters (**8-20**) nor for the flavan-3-ols (**1–7**).

For flaven-3-ols, it was observed that esterification with gallic acid at C-3-enhanced binding to caffeine [1]. In the present investigation it has been demonstrated that the C-3 galloyl ester CG (**3**) had higher binding activity on 5HT1, opiate and dopamine 1 receptors more strongly than that of compound with C-3OH (e.g. **1**, Table 2). Similarly, it was noted that the C-3 galloyl ester EGCG (**7**) inhibited binding of radioligands to opiate and dopamine 1 receptors more than did EGC (**6**). However, the converse has been observed for both sets of these compounds since CA (**1**) inhibited binding of radioligand to β-adrenoceptor more than did CG (**3**) and EGC (**6**) inhibited binding of ligands to 5HT1 and dopamine 2 receptors more strongly than did EGCG (**7**). It appeared that there was no simple correlation between mono-, di- and trihydroxy substituents in the B rings of monomeric flavan-3-ols as exemplified by EAF (**4**) and EC (**5**) with strong inhibition of ligand binding to β-adrenoceptor in contrast to EGC (**6**), whereas the reverse applied when the 5HT 1 receptor results were considered (Table 2).

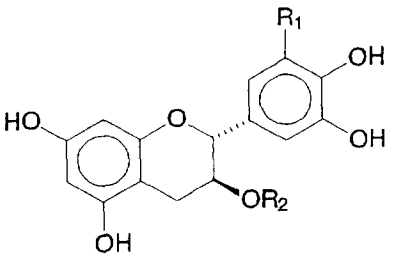
Table 3. Phenolic compounds ($10^{-5}$ M) with more than 60% inhibition of binding (3+, 4+) in radioligand receptor binding assays*

| Receptor | Active compounds† |
|---|---|
| α1-Adrenoceptor | PB-4 |
| α2-Adrenoceptor | 4TGG, PB-4, RD |
| β-Adrenoceptor | EAF, EC, 2TGG, 3TGG, 2HHDP |
| Dopamine 1 | CG, Ger |
| Dopamine 2 | 4TGG |
| 5HT1 | EGC, PB-2, PB-3, PB-4 |
| Opiate | CG, 2DGG, 3TGG, PGG, Dav |

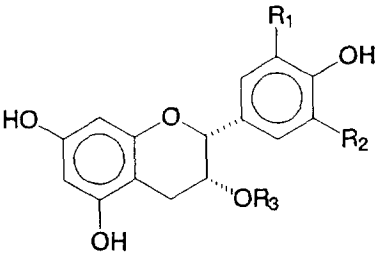
*Data taken from Table 2; †for abbreviations, see Table 1.

The dimeric procyanidins B-2 (**8**), B-3 (**9**) and B-4 (**10**) were equally effective as strong inhibitors of binding (>80%, 4+) of radioligand to the 5HT1 receptor under the test conditions used. This finding indicated that the configurational difference at C-3 and C-3′ of the hydroxyl groups and the C-4 to C-8 linkage did not play any important role for this particular receptor. However, procyanidin B-4 (**10**) showed specificity in binding to the α1-adrenoceptor (3+) in contrast to compounds **8** and **9** which had only weak inhibition of binding activity.

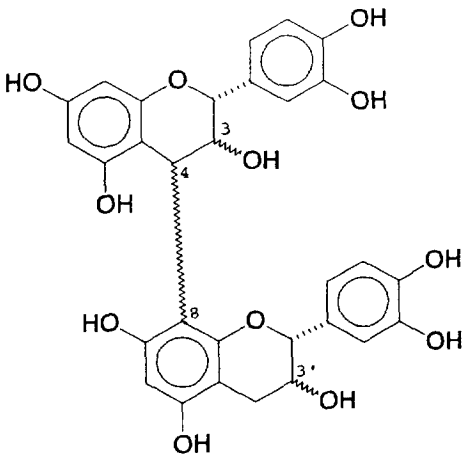
DGG (**11**) with two galloyl ester functions exhibited strong inhibition of binding of radioligand to the opiate receptor (>60%, 3+), moderate activity (2+) for inhibition of binding to the 5HT1 receptor with only weak or no activity for the remaining 14 receptors. In contrast, 2TGG (**12**) and 3TGG (**13**), both with 3-galloyl ester functions, showed no inhibition of binding to the 5HT1 receptor. However, both compounds **12** and **13** showed inhibition of radioligand binding to the β-adrenoceptor (3+) and to the opiate receptor (2+, 3+, respectively). Whilst 4TGG (**14**) and PGG (**15**) with 4 and 5 galloyl ester functions, respectively, failed to inhibit binding to the β-adrenoceptor (+, ±, respectively) but were effective at the α-2-adrenoceptor (3+, 2+, respectively). 4TGG (**14**) was the most active of the 20 phenols tested in its ability to inhibit binding to the dopamine 2 receptor (Table 2).



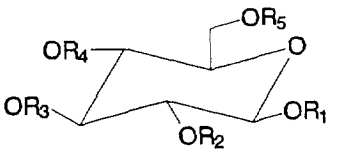

**1-3**



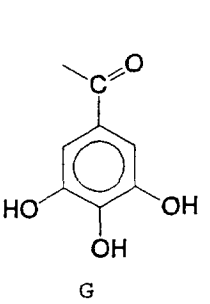

**4-7**



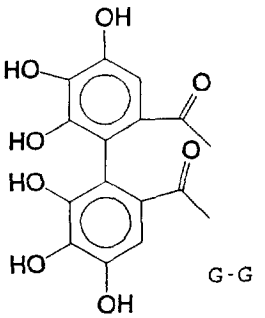

**8-10**



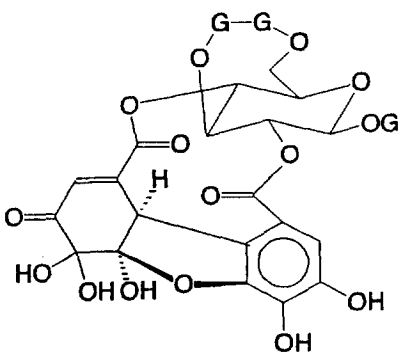

**11-18**



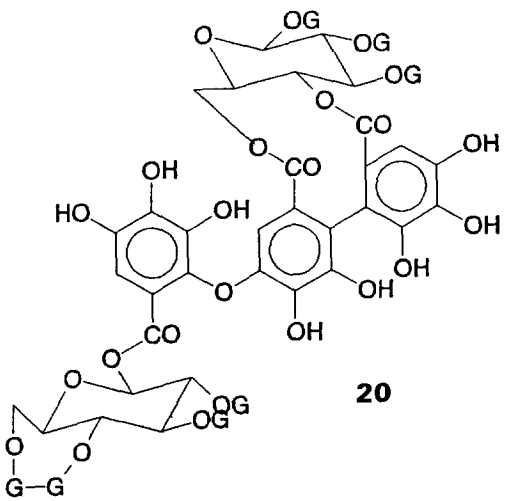

G




G-G




**19**




**20**

Surprisingly, 1HHDP (**16**) failed to inhibit ligand–receptor binding for 15 ( < + ) of the 16 receptors (only 2+ to opiate receptor). These findings contrast with the structurally closely related compound 2HHDP (**17**) which markedly inhibited ligand binding to the $\beta$-adrenoceptor. Again this finding contrasts with anticipated results which are based on protein–tannin binding studies where hexahydroxydiphenic acid moieties bind less strongly than correspondingly substituted digalloyl moieties [1]. Davidiin (**18**) is closely related to PGG (**15**), both being pentasubstituted glucose, the former possessing a 1,5-hexahydroxy-diphenoic acid moiety and latter 1,5-digalloyl ester groups. No significant differences in their receptor binding activities could be attributed to these chemical differences as compounds (**15**) and (**16**) were broadly similar in their actions (Table 2).

Geraniin (**19**) has similar structure to davidiin (**18**)

differing in one important respect, namely the presence of a dehydrohexahydroxy-diphenic acid moiety in (**19**). The activity of (**19**) differs from that of (**18**) in that the former was not able to inhibit, to the same extent, binding of ligands to the $\beta$-adrenoceptor or opiate receptor (Table 2). However, geraniin (**19**), in contrast to davidiin (**18**), was able to inhibit ligand binding to the dopamine 1 receptor. Rugosin D (**20**) was the highest molecular weight compound ($M_r$ 1874) tested and it exhibited strong inhibition of ligand binding to only the $\alpha$2-adrenoceptor. In contrast to davidiin (**18**), rugosin D (**20**) showed only weak ability to inhibit ligand binding to the opiate receptor (Table 2).

The $IC_{50}$ values of four selected phenolic compounds against three of the 16 receptors are shown in Table 4. These results again illustrate the difficulties encountered in attempting to assess the ability of phenolic compounds to inhibit binding of radioligands to receptors. CG (**3**) showed weaker activity with an $IC_{50}$ value of 36 nM in the test with the opiate receptor. This result contrasted with the 3+ activity given in Table 2 and with the $IC_{50}$ value of 1.0 nM for the control compound naloxone (Table 4). PB-3 (**9**) had an $IC_{50}$ value of 0.13 nM for the 5HT1 receptor showing greater potency than the radioligand 5HT which had an $IC_{50}$ value of 2.0 nM; it was assessed as 4+ in its activities shown in Table 2. PB-4 (**10**) and rugosin D (**20**) have $IC_{50}$ values of 2.13 and 8.40 nM, respectively, for the $\alpha$2-adrenoceptor showing weaker activity in comparison to the specific ligand UK14304 which had an $IC_{50}$ value of 0.06 nM. Compounds **10** and **20** were rated as 3+ in the comparison of activities which are given in Table 2.

Radioligand–receptor binding assays play an important role in the screening programmes of the pharmaceutical industry for novel drug entities. Many thousands of natural products and plant extracts are subjected to radioligand–receptor binding assay screens in the search for novel drug entities with selective action. The aim is to discover compounds which act at specific receptors and which show no activity for related subsets or related receptors. Because of their ability to bind with protein, tannins are frequently removed from extract prior to screening procedures by the addition of polyvinyl pyrrolidine [8].

Table 4. $IC_{50}$ values* of selected phenolic compounds and ligands for specific receptors

| Compound | Receptor† | $IC_{50}$ (nM) ± S.E.M. |
|---|---|---|
| CG (**3**) | Opiate | 36 ± 0.03 |
| PB-3 (**9**) | 5HT1 | 0.13 ± 0.01 |
| PB-4 (**10**) | $\alpha$2-Adrenoceptor | 2.13 ± 0.018 |
| RD (**20**) | $\alpha$2-Adrenoceptor | 8.48 ± 0.05 |

* The $IC_{50}$ values are the means of six determinations.

† The $IC_{50}$ values of positive control compounds were naloxaone 1.0 nM for opiate receptor; 5HT 2.0 nM for 5HT1 receptor; UK 14304 0.06 nM for $\alpha$2-adrenoceptor.

Such an approach, although justifiable from the industrial viewpoint, may well result in the failure to detect biologically active tannins. It is known that tannins and smaller phenolic compounds have a wide range of biological actions including antitumour, antiviral, anti-HIV, antimutagenic, antioxidant, antihypersensitive, lipid cholesterol lowering [1, 4, 6, 9–11] and anticonvulsant [12]. Whereas, certain tannins such as acetonylderaniin were found to reverse the fall in arterial blood pressure in conscious hypertensive rats induced by hexamethonium [13]. Some studies indicated that enzymes can be inhibited by tannins selectively [14, 15]. Three classes of chemically defined tannins, gallotannins, ellagitannins and condensed tannins were examined for the inhibitory activities against poly (ADP-ribose) glycohydrolase [16]. The results demonstrated that ellagitannins showed higher inhibitory activities than that of gallotannins and condensed tannins were not appreciably inhibitory. Ong *et al.* [17] also reported the similar finding that tannic acid (a hydrolysable tannin) selectively inhibited insulin-stimulated lipogenesis. However, its monomer, gallic acid, did not show such activity. In a binding assay, it was observed that the specific binding of insulin to its receptor was not inhibited by tannic acid in the concentration range 0–200 $\mu$M, whereas insulin-stimulated autophosphorylation of the insulin receptor and receptor-associated tyrosine kinase phosphorylation of RR-SRC peptide were inhibited by tannic acid at concentration as low as 25 $\mu$M.

The present study has revealed that for 20 phenolic compounds which were assessed for their ability to inhibit binding of specific radioligands to 16 receptors, that there is a wide range of differing activities. Furthermore, some compounds, under the test conditions utilized, have the ability to inhibit ligand binding to specific receptors. Hence, the removal of tannins from plant extracts prior to screening would result in missing biologically active compounds and possibly new drug entities. This had to be balanced against the large number of 'false-positive' results which will be thrown up by common phenolic plant constituents in industrial screening programmes. In addition to the needs of industry, there is also the need to investigate in more detail the active principles of medicinal plants which are taken orally as complex mixtures of plant constituents in herbal medicines. Our findings show that some phenolic compounds including tannins, do show specific activities at the receptor level and that these cannot be explained solely in terms of protein binding. Tannin–protein interactions are influenced by characteristics of the tannin (including size, structure) and of protein (including size, amino acid composition) and conditions of reaction (pH, temperature, solvent, time) [18]. The pH dependence of protein precipitation by condensed tannins suggests that the phenolic hydroxyl acts as a hydrogen bond donor and the carbonyl peptide bond serves as the hydrogen bond acceptor. Hydrogen bonding is augmented by hydrophillic interactions between non-polar domains

Table 5. Radioligand receptor-binding methods

| Receptor | Ligand (conc. nM) | Ligand $K_d$ values (nM) | Control (conc. M) | NSB* (conc. M) | Inc.* (min)/°C | Buffer† | Tissue§ |
|---|---|---|---|---|---|---|---|
| α1-Adrenoceptor | Prazosin (0.2) | 0.2–0.3 | Prazosin ($10^{-7}$–$10^{-11}$) | Phentolamine ($10^{-5}$) | 30/25 | 50 mM Tris | Rat brain |
| α2-Adrenoceptor | UK14304 (0.5) | 0.4–0.8 | Phentolamine ($10^{-6}$–$10^{-10}$) | Phentolamine ($10^{-5}$) | 30/25 | 50 mM Tris | Rat brain |
| β-Adrenoceptor | DHA‡ (2.0) | 1.5–4.5 | Propranolol ($10^{-6}$–$10^{-10}$) | Propranolol ($10^{-6}$) | 30/25 | 50 mM Tris + 10 mM $MgCl_2$ | Pig brain |
| Adenosine 1 | CHA (2.0) | 1.2–3.8 | CHA ($10^{-6}$–$10^{-10}$) | CHA ($10^{-6}$) | 60/25 | Krebs 50 mM Tris | Rat brain |
| Dopamine 1 | SCH-23390 (0.5) | 0.2–0.6 | SCH-23390 ($10^{-6}$–$10^{-10}$) | (+)-butaclamol ($10^{-5}$) | 30/25 | 50 mM Tris + 2 mM $MgCl_2$ | Pig brain striatum |
| Dopamine 2 | Spiperone (0.2) | 0.1–0.3 | Butaclamol ($10^{-7}$–$10^{-11}$) | Butaclamol ($10^{-6}$) | 30/25 | 50 mM Tris | Pig brain striatum |
| Muscarinic | QNB (0.2) | 0.1–0.3 | Atropine ($10^{-6}$–$10^{-10}$) | Atropine ($10^{-6}$) | 60/25 | Krebs Tris | Pig brain |
| $Ca^{2+}$DHP | Nitrendipine | 0.1–0.4 | Nitrendipine ($10^{-7}$–$10^{-11}$) | Nimodipine ($10^{-7}$) | 30/25 | Krebs Tris | Pig brain |
| $K^+$ATP | Glibenclamide (1.0) | 0.7–1.5 | Glipizide ($10^{-7}$–$10^{-11}$) | Glibenclamide ($10^{-5}$) | 30/25 | Krebs Tris | Pig brain |
| 5HT1 | 5HT (2.0) | 1.5–4.5 | 5HT ($10^{-5}$–$10^{-10}$) | 5HT ($10^{-5}$) | 30/25 | 5HT buffer | Guinea pig brain |
| 5HT1A | 8-OH DPAT (1.0) | 0.8–1.8 | 8-OH DPAT ($10^{-6}$–$10^{-10}$) | 8-OH DPAT ($10^{-6}$) | 30/25 | 5HT buffer | Guinea pig brain |
| 5HT2 | Ketanserin (2.0) | 1.5–4.0 | Spiperone ($10^{-6}$–$10^{-10}$) | Spiperone ($10^{-6}$) | 30/25 | 50 mM Tris | Rat brain |
| Histamine 1 | Pyrilamine (1.0) | 0.8–1.5 | Pyrilamine ($10^{-6}$–$10^{-10}$) | Promethazine ($10^{-6}$) | 60/25 | 50 mM Tris | Pig brain |
| Benzodiazepine | Flunitrazepam (1.0) | 0.9–2.8 | Flunitrazepam ($10^{-6}$–$10^{-10}$) | Diazepam ($10^{-5}$) | 60/5 | 50 mM Tris | Pig brain |
| Opiate | Naloxone (1.0) | 0.8–2.0 | Naloxone ($10^{-6}$–$10^{-10}$) | Naloxone ($10^{-5}$) | 30/25 | 50 mM Tris | Pig brain |
| $Na^+/K^+$ATPase | Ouabain (5.0) | 4.0–7.0 | Ouabain ($10^{-5}$–$10^{-9}$) | ATP buffer 2** | 60/37 | ATP buffer 1** | Rat brain |

* NSB: compound for determination of non-specific binding; INC: incubation time and temperature.

† Buffer: 50 mM Tris: 50 mM Tris HCl in distilled water. Krebs Tris: 50 mM Tris HCl, 136 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$ in distilled water. 5HT buffer: 50 mM Tris HCl, 2 mg pargyline, 4 ml 2.5 mM $CaCl_2$ and 1 g ascorbic acid in 1 litre distilled water. ATP buffer 1 (assay buffer): 50 mM Tris HCl, 300 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris-ATP (vanadium-free, Sigma A0520) in distilled water. ATP buffer 2 (NSB buffer): 50 mM Tris HCl, 300 mM NaCl, 10 mM $MgCl_2$ in distilled water. All buffers were adjusted to pH 7.5 at 25°C.

‡ DHA: dihydroalprenolol, CHA: $N^6$-cyclohexyladenosine, QNB: quinuclidmyl benzilate, DHP: dihydropyridines, 5HT: serotonin, 8OH-DPAT: 8-hydroxy dipropylaminotetralin.

§ Tissue preparation: whole rat and pig brains and pig striatum dissected out from the brains were used. The tissues were homogenized in 10 volumes of ice-cold Tris HCl buffer (50 mM) for 20 seconds. Homogenates were centrifuged for 20 min at 50 000 *g* in a Sorvall centrifuge (RC 5C Centrifuge SS-34 rotor). The supernatant was discarded and the pellet reconstituted in fresh ice-cold buffer (10 volumes). The suspension was recentrifuged under the same conditions, on two further occasions, discarding the supernatants. The final pellet was homogenized for 20 s in 10 volumes of ice-cold Tris HCl buffer and the homogenate was stored in aliquots at −70°C for subsequent study. Guinea-pig brain was treated as above with the following modifications: the suspension was incubated at 37 for 10 min before the final spinning and the storage buffer (Tris HCl, 50 mM) contained 4.0 mM $CaCl_2$, 1.0 g/l ascorbic acid and 10 μM pargyline.

of certain proteins and the non polar regions of tannin molecules [18]. It is known that tannin–protein interactions are characterized by some specificity [18, 19] and in tannin/glycoprotein complexation the oligosaccharide present in glycosylated proline-rich proteins enhance both the affinity and the selectivity of binding [20]. Further investigations are required in order to reveal the specificity of tannin–receptor interactions.

## EXPERIMENTAL

*Phenolic compounds tested.* Compounds (**1–8**) and (**12–20**) were isolated by one of us (Y.C.) during Ph.D. studies under the direction of Professor E Haslam, University of Sheffield [21]. Compounds (**9–11**) were isolated from *Croton lechleri* [22].

*Radioligand receptor binding assays.* The receptors utilised and ligands are tabulated in Table 5. The general procedure is as follows. Each test compound (50 $\mu$l, concentration of $10^{-5}$M) and required [$^3$H]-ligand (50 $\mu$l at specified concentration given in Table 5) were mixed with homogenate containing required receptor (400 $\mu$l, final protein concentration of 0.5 mg/ml). The mixture was incubated for a specified time at a specified temperature (Table 5) and then filtered on a pressure reduced Brandel Cell Harvester (Brandel, Gaithorsberg, MD) using GF/B filters. The filters covered with samples were punched into vials and soaked in scintillation solution for at least 1 hr before counting. The scintillation counters (LS-6000 TA, Beckman Ltd) gave sample counts in DPM.

*Analysis of ligand–receptor binding data.* Non-specific binding was estimated in the presence of a high concentration of a receptor specific non-radioactive compound (Table 5, NSB). The amount of ligand specifically bound was determined by subtracting the amount of ligand non-specifically bound from the total amount of radioactivity bound in the absence of any compound and expressed as a percentage of the total binding.

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