Full Paper

Synthesis and Antioxidant and Antitumor Activity of Novel Pyridine, Chromene, Thiophene and Thiazole Derivatives

Ahmed A. Fadda, Moged A. Berghot, Fathy A. Amer, Doria S. Badawy, and Nesma M. Bayoumy

Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt

2-Tosylacetonitrile (**1**) when reacted with *α,β*-unsaturated nitriles **2a–c** or a mixture of formaldehyde and 3-amino-2-substituted-pent-2-endinitriles **6a**,**b** yielded pyridine derivatives **3a–c** and **9a**,**b**, respectively, while when subjected to react with salicylaldehyde yielded chromene derivatives **4** and **5**, subsequently. The behavior of thiocarbamoyl derivative **10** derived from **1** towards some α-halogenated compounds have been investigated as well as its behavior towards elemental sulfur and phenyl isothiocyanate. Newly synthesized compounds were screened for their antioxidant activity, erythrocytes haemolysis and bleomycin-independent DNA damage. Some of the tested compounds exhibited promising activities.

Keywords: 2-Tosylacetonitrile / Arylidenes / Antioxidant and antitumor activities / α-Halo compounds / Thiocarbamoyl

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Introduction

This work is the continuation of a program aiming to develop new simple methods for the synthesis of functionally substituted heterocycles with anticipated biological activity. We have recently extended it to include the investigation of the pharmacological aspects of the newly synthesized heterocycles based on the finding that some heterocycles can achieve activity in both the pharmacological and pesticidal areas, e.g. thiabenzdazole, the well known human and veterinary anthelmintic, is also used as a fungicide [1, 2].

Results and discussion

Chemistry

Pyridines and coumarin derivatives have recently received considerable attention due to their synthetic and pharmaceutical importance and different approaches for their synthesis have been developed [3–5]. In the last few years the authors have been exploring the synthetic potential, scope and limitations of activated nitriles in heterocyclic synthesis [6–8]. Several new approaches for the synthesis of five and six membered rings and their fused heterocyclic derivatives have been developed during this work [9, 10]. In the present work we explore the synthetic potential of 2-tosylacetonitrile (**1**) to form poly-substituted pyridines and coumarin heterocycles *via* the reaction of **1** with *α,β*-unsaturated nitriles **2a–c** or salicylaldehyde, respectively. Thus, compound **1** reacted under triethylamine (TEA) catalysis with *α,β*-unsaturated nitriles **2a–c** in refluxing ethanol to afford 6-amino-4-substituted-5-tosylnicotinonitriles **3a–c** [11]. The IR spectra of these products showed in each case absorption bands at ν 3337, 2257 and 1610 cm^{-1} corresponding to NH_2, CN and C=N group, respectively. Structures **3a–c** were assigned to these products on the basis of the spectral as well as analytical data. The ^{1}H-NMR spectrum of **3a** revealed a broad signal at *δ* 6.5 ppm assigned to the amine protons and a multiplet at *δ* 7.8–8.4 ppm assigned to the pyridine-2H and aromatic protons.

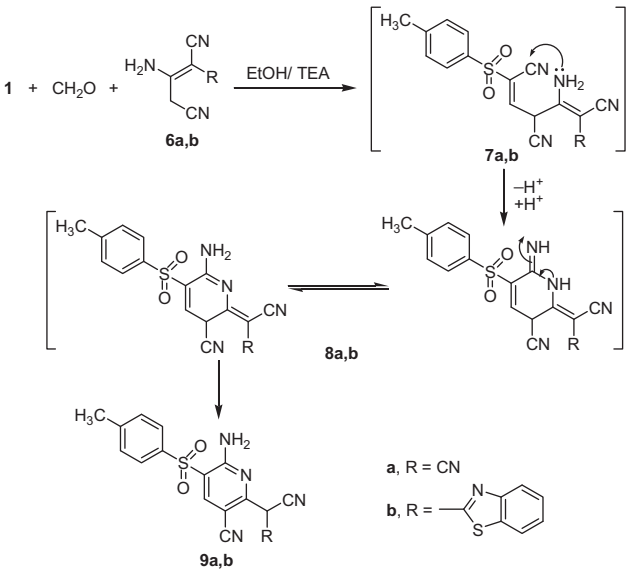
Compound **1** reacted with salicylaldehyde in refluxing ethanol catalyzed by TEA to give 3-tosyl-2*H*-chromen-2-imine (**4**) in high yield. Mass spectral measurements and analytical data were in complete agreement with structure **4** which showed the molecular ion peak at *m/z* 300 (M^++1). Moreover, the resulting coumarin derivatives have latent functional substituents which have the potential for further chemical transformations giving new routes for the preparation of substituted coumarin derivatives with possible biological

Correspondence: Prof. Ahmed A. Fadda, Department of Chemistry, Faculty of Science, Mansoura University, El-Gomhoria Street, Mansoura, 35516, Egypt.
E-mail: afadda2@yahoo.com
Fax: +20502246781

activity. 3-Tosyl-2*H*-chromen-2-one (**5**) was synthesized *via* hydrolysis of **4** in a mixture of conc. hydrochloric acid and ethanol (Scheme 1). The mass spectrum of **5** showed the molecular ion peak at *m/z* 300 (M^+) which is in agreement with the expected molecular formula $C_{16}H_{12}O_4S$.

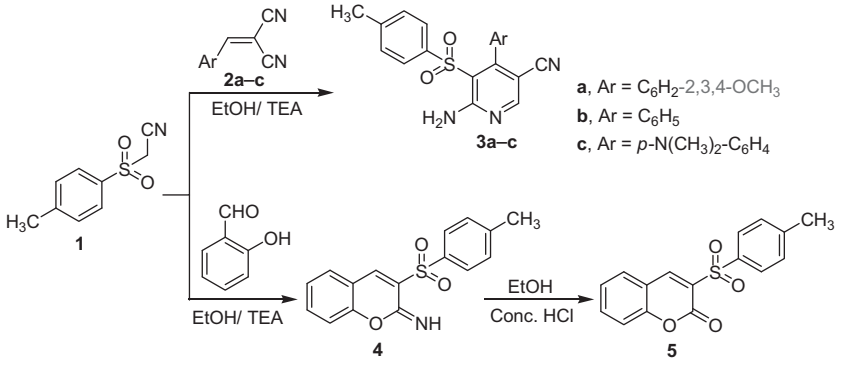
Aldehydes condense with active methylene nitriles to yield the corresponding ylidene derivatives [12]. The reaction of these ylidenes with active methylene and active methyl reagents has been extensively utilized for the synthesis of 4-pyrans, pyridines and benzene derivatives. Although α-functionally substituted acrylonitriles are expected to react similarly with active methylene reagents affording substituted pyran, pyridine and benzene derivatives, reactions of this type have not been reported, to our knowledge, with the exception of our recent report [13]. The difficulty of preparing α-functionally substituted acrylonitriles might explain the lack of reports on their utility. In the present paper we report the results of our work aiming at an investigation of the synthetic potentialities of this kind of reagent and related functionally substituted acrylonitriles.

It was found that compound **1** reacts with a mixture of formaldehyde and 3-aminoprop-1-en-1,1,3-tricarbonitriles **6a**,**b** in boiling ethanol containing a catalytic amount of TEA to give 6-amino-2-(substituted (cyano)methyl)-5-tosylnicotinonitriles **9a**,**b**. Structure **9a** was established by elemental and spectroscopic analysis. The IR spectrum of **9a** showed absorption bands at υ 3450, 2207 and 1628 cm^{-1} corresponding to NH_2, CN and C=N groups, respectively. The mass spectroscopic and analytical data were in agreement with structure **9a** (*c.f.* Experimental Part). The formation of **9a** is assumed to proceed *via in situ* formation of the methylene derivative of the nitrile **1** which was added to the dimer **6a** yielding the intermediate Michael adduct **7**. These were spontaneously cyclized to the intermediates **8a**,**b** giving the sensitive product **9a**. In the same way, **1** can react with a mixture of formaldehyde and **6b** to yield the pyridine derivative **9b**. The structure of the product **9b** was established as described above for **9a** (Scheme 2).

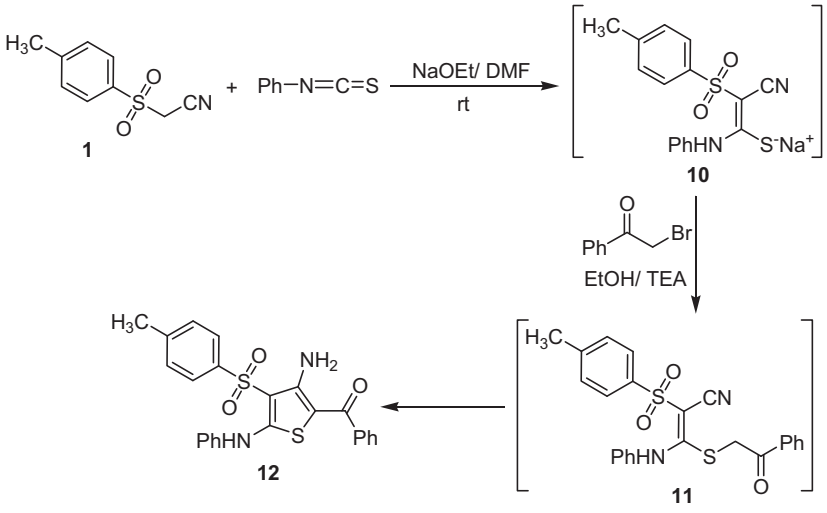


Scheme 2. Synthesis of 6-amino-2-(substituted (cyano)methyl)-5-tosylnicotine-nitriles **9a**,**b**.

Previously, we have investigated the reaction of phenyl isothiocyanate with active methylene compounds in alkaline medium, which has been proved to be a convenient route for the synthesis of thiazole, pyrazole, oxazine and pyrimidine ring systems [14]. Now, we have extended our synthetic program to the synthesis of otherwise inaccessible heterocyclic ring systems utilizing phenyl isothiocyanate as a key starting material. It is known that a great variety of reactants bearing the N=C=S fragment undergo cyclization on reaction with α-halocarbonyl compounds to afford thiazoles, 2,3-dihydrothiazoles and thiazolidines [15], which have been shown to exhibit local anaesthetic [16], antiprotozoal [17] and fungicidal properties [18]. In this paper, we describe a generally applicable extension of this synthetic approach, first



Scheme 1. Reactions of 2-tosylacetonitrile (**1**) with arylidenes and salicylaldehyde.



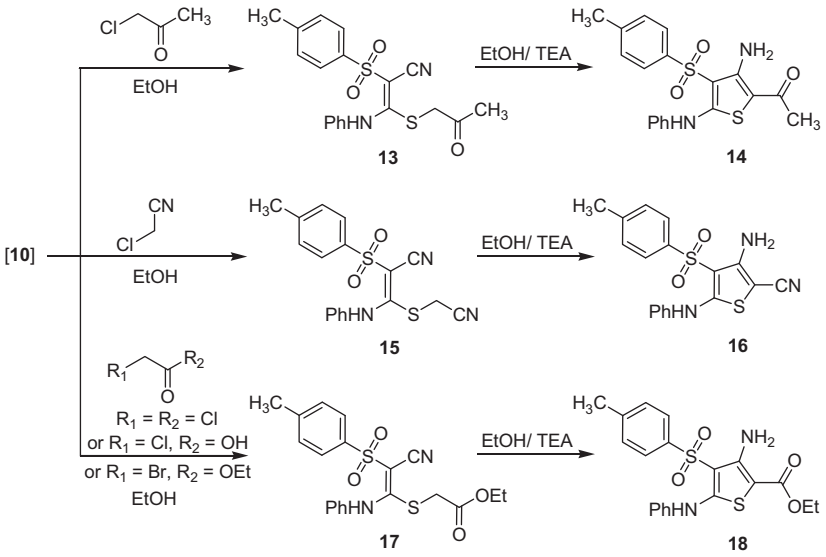
Scheme 3. Synthesis of 3-tosylthiophene derivative **12**.

reported by Hantzsch and Weber [19]. Thus, the base-prompted reaction of the acidic methylene compound **1** with phenyl isothiocyanate in dry dimethylformamide (DMF) at room temperature yields the non-isolable intermediate **10**. The intermediate **10** undergoes *in situ* cyclization upon the reaction with equimolar amounts of phenacyl bromide in boiling ethanol containing a catalytic amount of TEA, giving product **12**. (3-Amino-5-(phenylamino)-4-tosylthiophen-2-yl)(phenyl)methanone (**12**) was suggested for this product on the basis of analytical and spectral data. The mass spectrum of **12** showed the ion peak at *m/z* 448 (M^+) which is in agreement with the molecular formula $C_{24}H_{20}N_2O_3S_2$. The reaction may occur through the non-isolated intermediacy of acyclic derivative **11**. Attempts to isolate **11** by refluxing phenacyl bromide with the intermediate **10** in ethanol were unsuccessful. Compound **12** is assumed to be formed *via* the acyclic intermediate **11** (Scheme 3).

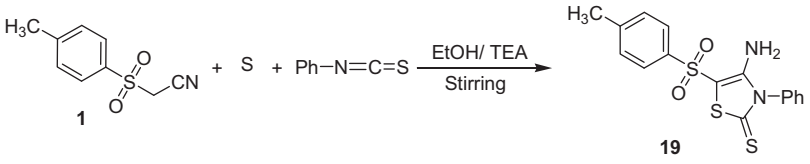
Compound **10** reacted readily with chloroacetone in boiling ethanol to afford the acyclic intermediate **13** by NaCl elimination. Refluxing **13** in ethanol with a catalytic amount of TEA gave the thiophene derivative **14** whose structure was confirmed by elemental analysis and spectral data (*c.f.* Experimental Part). The structure of **14** was further confirmed by its alternative synthesis. Thus, refluxing **10** with chloroacetone in DMF affords the thiophene derivative **14** in a good yield. Similarly, when the intermediate **10** was treated with chloroacetonitrile in refluxing ethanol containing a catalytic amount of TEA, the corresponding acyclic intermediate **15** is exclusively isolated in good yield. The structure **15** was confirmed on the basis of elemental and spectral data, *e.g.* the IR spectrum exhibits bands at υ 3200 (NH), 2220, 2195 cm^{-1} (2 CN). Its ^{1}H-NMR spectrum indicated a CH_2 signal at δ 3.23 ppm. Furthermore, heating of the intermediate **15** in ethanol containing a catalytic amount of TEA afforded the thiophene derivative **16**. Structure **16** was established based on its IR spectrum which showed bands related to NH_2, NH and CN functions. Its H-NMR spectrum revealed a multiplet signal at δ 7.31–7.56 ppm (9H, aromatic), broad signals at δ 6.5 (2H, NH_2) and δ 8.7 ppm (1H, NH). On the other hand, it was found that it is directly formed by refluxing **10** and chloroacetonitrile in DMF.

When **10** was treated with an equimolar amount of chloroacetyl chloride or with chloroacetic acid in boiling ethanol, a product that analyzed for $C_{20}H_{20}N_2O_4S_2$ was isolated in each case in good yield. The acyclic structure **17** was established based on its IR spectrum that showed bands related to NH, CN and CO function groups. Its H-NMR spectrum revealed a muliplet at (δ ppm) 7.50–8.56 (9H, aromatic), a triplet signal at δ 1.3 (3H, CH_3), singlet at δ 1.9 (3H, CH_3), singlet at δ 3.7 (2H), quartet at δ 4.3 (2H, CH_2) and a D_2O exchangeable NH at 8.91 ppm. Alternatively, treatment of **10** with ethyl bromoacetate in refluxing ethanol gave a single product, which is identical in all respects to **17** (m.p., mixed m.p. and IR spectrum). The mass spectrum of **17** showed the molecular formula $C_{20}H_{20}N_2O_4S_2$ ($M^+ = 416$). Refluxing of **17** in ethanol with a catalytic amount of TEA afforded the corresponding thiophene derivative **18** (Scheme 4).

The thiazoline heterocyclic ring incorporating a tosyl moiety was prepared from compound **1**. Thus, treatment of **1** with a mixture of sulfur metal and phenyl isothiocyanate in ethanol and excess of TEA and stirring overnight gave the corresponding *N*-phenylthiazole derivative **19** that is in complete agreement with mass spectral measurements and analytical data (Scheme 5). The structure of **19** could be established for the reaction product based on the IR spectrum which showed absorption bands at υ 3480 and 3390 cm^{-1} (NH_2). Its ^{1}H-NMR spectrum revealed a D_2O exchangeable



Scheme 4. Synthesis of thiophene derivatives **14**, **16** and **18**.



Scheme 5. Synthesis of 4-amino-3-phenyl-5-tosylthiazole-2(3*H*)-thione (**19**).

proton at δ 6.1 ppm due to NH_2 protons besides a multiplet at δ 7.2–8.1 ppm due to 9 aromatic protons.

Pharmacology

Antioxidant activity for ABTS and erythrocytes haemolysis

All compounds were tested for antioxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and the rate of erythrocyte haemolysis. The pro-oxidant activities of the compounds were assayed for their antioxidant effects using ABTS assay. Compounds **12** and **19** proved to exhibit potent antioxidative activity. On the other hand, compounds **14**, **16**, and **18** showed a very good activity. In the meantime, compounds **1**, **3a**, **3c**, **9a** and **9b** showed moderate activity, while compounds **3a**, **12**, **18** and **19** showed high activity in case of erythrocyte haemolysis.

From Table 1 and the above-mentioned results, we may include the following structure activity relationships (SARs) and by comparing the tested compounds: (1) The presence of thiophene and thiazole ring systems incorporated with the tosyl moiety enhances the activity. (2) Introducing acyclic chains or pyridine moieties into the tosyl moiety did not affect the antioxidant activity (ABTS method). (3) Introducing the pyridine moiety incorporating three methoxy groups (electron donating groups) enhances the activity in erythrocytes haemolysis (Fig. 1).

Table 1. Antioxidant assay for the prepared new compounds.

Compd. no.	ABTS (Inhibition %)	Erythrocytes haemolysis (%)
Control	–	–
L-Ascorbic acid	88.61	0.85
1	39.70	1.40
3a	41.60	0.86
3c	35.55	5.55
9a	40.90	1.50
9b	38.20	3.65
12	83.30	0.95
14	72.30	1.10
16	76.20	1.90
18	78.20	0.86
19	80.30	0.85

Bleomycin-dependent DNA damage

The bleomycins are a family of antitumor antibiotics, which are used routinely as antitumor agent. The bleomycin assay has been adopted for assessing the pro-oxidant effect of food antioxidants. The antitumor antibiotic bleomycin binds iron

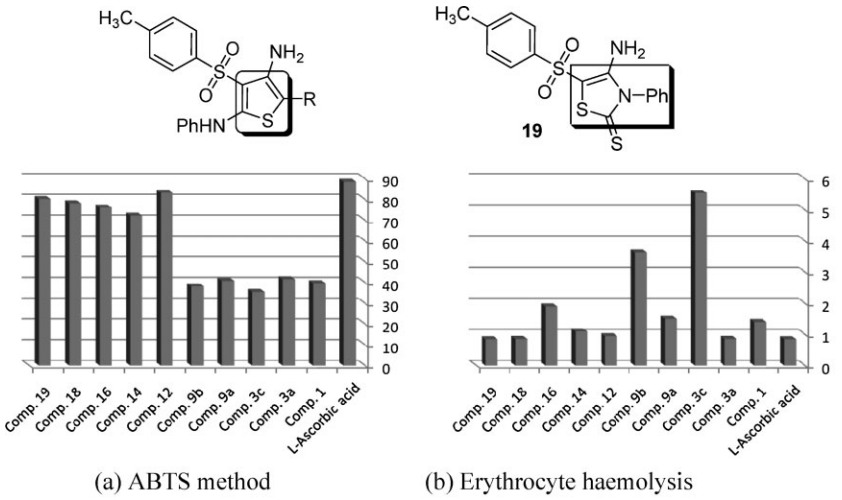


Figure 1. Antioxidant activity screening assay.

ions and DNA. The bleomycin-iron complex degrades DNA that, upon heating with thiobarbituric acid, yields a pink chromogen. Added suitable reducing agent ''antioxidants'' compete with DNA and diminish chromogen formation. We select the best results of antioxidant activity for ABTS, erythrocytes haemolysis and evaluate their bleomycin dependent-DNA damage. Among the tested compounds (Table 2), compound **12** showed the highest protection activity against DNA damage induced by bleomycin-iron complex, thus diminishing chromogen formation between the damaged DNA and thiobarbituric acid (TBA).

Table 2. Result of bleomycin-dependent DNA damage assay of compound **1** as blank and compound **12** as the best antioxidant agent.

Compd. no.	Absorbance
Vit. C	0.100
1	0.196
12	0.088

Experimental

Chemistry

All melting points are uncorrected in degree centigrade and determined on Gallenkamp electric melting point apparatus. The infrared (IR) spectra were recorded (KBr disk) with a Mattson 5000 FTIR spectrometer at the Faculty of Science, Mansoura University. The ^{1}H-NMR spectra were determined on a Bruker WPSY 200 MHz spectrometer with TMS as internal standard and the chemical shifts are in δ ppm using dimethylsulfoxide (DMSO) as solvent. The mass spectra were recorded at 70 eV with a Varian MAT 311 at the Microanalytical Center, Faculty of Science, Cairo University. Elemental analyses (C, H and N) were carried out at the Faculty of Science, Cairo University, the results were found to be in good agreement (±0.03%) with the calculated values.

*General procedure for the synthesis of 6-amino-4-substituted-5-tosylnicotinonitrile derivatives **3a–c***

A mixture of 2-tosylacetonitrile (**1**) (1.95 g, 0.01 mol) and α,β-unsaturated nitriles, namely **2a** (2.44 g, 0.01 mol) or **2b** (1.54 g, 0.01 mol) or **2c** (1.97 g, 0.01 mol) in ethanol (15 mL), containing a catalytic amount of TEA (0.4 mL) were refluxed for 6 h. The solid products obtained after cooling were filtered off, dried and recrystallized from ethanol to give pyridine derivatives **3a–c**, respectively.

*6-Amino-5-tosyl-4-(2,3,4-trimethoxyphenyl)nicotinonitrile (**3a**)*

White crystals, yield 80%, mp: 90°C, IR (KBr): ν_{max}, cm^{-1}: 3350–3450 (NH_2), 2220 (CN), 1610 (C=N), 1210, 1230 (SO_2); ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 7.1 (d, 2H, NH_2, exchangeable with D_2O), 7.7–8.0 (m, 6H, Ar–H), 8.5 (s, 1H, O-py-H). MS (*m/z*, %): 439 (M^+, 11.2%), 378 (100.0), 217 (64.7), 203 (15.4), 155 (30.5), 86 (49.2). Anal. Calcd. for $C_{22}H_{21}N_3O_5S$ (439.48): C, 60.12; H, 4.82; N, 9.56%. Found: C, 60.20; H, 4.87; N, 9.63%.

*6-Amino-4-phenyl-5-tosylnicotinonitrile (**3b**)*

White crystals, yield 75%, mp: 143°C, IR (KBr): ν_{max}, cm^{-1}: 3366 (broad, NH_2), 3030 (CH), 2217 (CN), 1615 (C=C), 1597 (C=N), 1255, 1208 (SO_2); ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 6.5 (s, 2H, NH_2), 7.8–8.4 (m, 5H, py-c_2-H+Ar). MS (*m/z*, %): 283 [M^+–(CN–C–CH=N), 14.6%], 219 (0.9), 195 (8.9), 155 (69.2), 128 (3.2), 91 (100.0). Anal. Calcd. for $C_{19}H_{15}N_3O_2S$ (349.41): C, 65.31; H, 4.33; N, 12.03%. Found: C, 65.38; H, 4.41; N, 12.11%.

*6-Amino-4-(4-(dimethylamino)phenyl)-5-tosylnicotinonitrile (**3c**)*

Orange crystals, yield 84%, mp: 220°C, IR (KBr): ν_{max}, cm^{-1}: 33361–3428 (NH_2), 2197 (CN), 1612 (C=N); ^{1}H NMR (DMSO-d_6): δ 1.2 (s, 3H, CH_3), 7.1–8.2 (m, 8H, Ar–H), 8.2 (s, 1H, py-C_2-H). MS (*m/z*, %): 392

(M^+, 100.0), 326 (52.2), 197 (60.2), 170 (42.3), 155 (63.4), 134 (43.7), 86 (100.0). Anal. Calcd. for $C_{21}H_{20}N_4O_2S$ (392.47): C, 64.27; H, 5.14; N, 14.28%. Found: C, 64.18; H, 5.09; N, 14.34%.

*Synthesis of 3-tosyl-2H-chromen-2-imine (**4**)*

A mixture of **1** (1.95 g, 0.01 mol) and salicyaldehyde (1.07 mL, 0.01 mol) in ethanol (15 mL) containing a catalytic amount of TEA (0.5 mL) was refluxed for 2 h. The product iminochromene **4** was obtained after cooling, filtered off, dried and recrystallized from ethanol. Orange crystals, yield 80%, mp: 190°C, IR (KBr): ν_{max}, cm^{-1}: 3344 (NH), 1211, 1148 (SO_2); ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 7.3–8.5 (m, 8H, Ar–H), 8.8 (s, 3H, NH). MS (*m/z*, %): 300 (M^++1, 1.12), 248 (0.96), 234 (100.0), 208 (9.4), 165 (4.1), 139 (5.0), 89 (32.1). Anal. Calcd. for $C_{16}H_{13}NO_3S$ (299.34): C, 64.20; H, 4.38; N, 4.68%. Found: C, 64.26; H, 4.41; N, 4.72%.

*Synthesis of 3-tosyl-2H-chromen-2-one (**5**)*

Compound **4** (2.99 g, 0.01 mol) was heated in a mixture of conc. HCl and ethanol (1:1, 20 mL) for 15 min. The reaction mixture was left to stand at room temperature overnight and the solid product was filtered off, dried and recrystallized from ethanol to give corresponding chromenone derivative **5**. Red crystals, yield 76%, mp: 220°C, IR (KBr): ν_{max}, cm^{-1}: 3036 (CH), 1740 (CO), 1212, 1151 (SO_2); ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 7.55–8.25 (m, 8H, Ar–H). MS (*m/z*, %): 300 (M^+, 3.0), 235 (97.0), 208 (33.7), 165 (10.8), 139 (15.1), 89 (100.0), 63 (61.5). Anal. Calcd. for $C_{16}H_{12}O_4S$ (300.33): C, 63.99; H, 4.03%. Found: C, 63.95; H, 3.98%.

*Synthesis of 6-amino-2-(substituted (cyano)methyl)-5-tosylnicotinonitriles **9a,b***

Equimolar amounts of **1** (0.78 g, 4 mmol), formaldehyde (1 mL, 30% aqueous solution) and 3-amino-2-substituted-pent-2-enedinitriles **6a** (0.53 g, 4 mmol) or **6b** (0.96 g, 4 mmol) in ethanol (25 mL) in the presence of a catalytic amount of TEA (0.4 mL). The reaction mixture was refluxed for 3 h and the obtained solid product while hot was filtered off, dried and recrystallized from ethanol.

*2-(6-Amino-3-cyano-5-tosylpyridin-2-yl)malononitrile (**9a**)*

Red crystals, yield 82%, mp: 200°C, IR (KBr): ν_{max}, cm^{-1}: 3450 (NH_2), 2207 (CN), 1628 (C=N); ^1H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 4.72 (s, 1H, CH), 6.43 (s, 2H, NH_2), 7.35–8.62 (m, 5H, Ar–H). MS (*m/z*, %): 337 (M^+, 65.2), 278 (76.1), 246 (52.6), 155 (38.0), 139 (100.0), 123 (58.9), 86 (65.2). Anal. Calcd. for $C_{16}H_{11}N_5O_2S$ (337.36): C, 56.96; H, 3.29; N, 20.76%. Found: C, 57.04; H, 3.36; N, 20.83%.

*6-Amino-2-(benzo[d]thiazol-2-yl(cyano)methyl)-5-tosylnicotinonitrile (**9b**)*

White crystals, yield 80%, mp: 290°C, IR (KBr): ν_{max}, cm^{-1}: 3358 (NH_2), 2190 (CN), 1601 (C=N); ^1H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 4.68 (s, 1H, CH), 6.47 (s, 2H, NH_2), 7.28–8.71 (m, 9H, Ar–H). MS (*m/z*, %): 445 (M^+, 61.7), 251 (100.0), 156 (23.9), 124 (16.3), 86 (61.7). Anal. Calcd. for $C_{22}H_{15}N_5O_2S_2$ (445.52): C, 59.31; H, 3.39%. Found: C, 59.37; N, 15.72%; H, 3.42; N, 15.79%.

*Preparation of sodium 2-cyano-1-(phenylamino)-2-tosylethenethiolate (**10**)*

To a stirred suspension of sodium ethoxide [prepared from 0.23 g, 0.01 mol sodium metal in 10 mL ethanol) in DMF (20 mL), 2-tosylacetonitrile (**1**) (1.95 g, 0.01 mol) was added. To the resulting solution the phenyl isothiocyanate (1.2 mL, 0.01 mol) was added and the reaction mixture stirred for 24 h at room temperature.

*Synthesis of (3-amino-5-(phenylamino)-4-tosylthiophen-2-yl)(phenyl)methanone (**12**)*

A mixture of equimolar amounts of **10** (0.01 mol) and phenacyl bromide (1.99 g, 0.01 mol) was refluxed in ethanol (20 mL) containing a catalytic amount of TEA (0.5 mL) for 6 h. The reaction mixture was cooled, filtered off, dried and recrystallized from ethanol to give **12**. Black powder, yield 78%, mp: 140°C, IR (KBr): ν_{max}, cm^{-1}: 3430 (NH_2), 3309 (NH), 1710 (CO); ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.5 (broad, 2H), 7.3–8.1 (m, 14H, Ar–H), 9.22 (s, 1H, NH). MS (*m/z*, %): 448 (M^+, 12.2), 164 (100.0), 120 (83.7), 86 (14.3). Anal. Calcd. for $C_{24}H_{20}N_2O_3S_2$ (448.56): C, 64.26; H, 4.49; N, 6.25%. Found: C, 64.33; H, 4.53; N, 6.30%.

*General procedure for the synthesis of the acyclic 3-(substituted-methylthio)-3-(phenylamino)-2-tosylacrylonitriles **13**, **15** and **17***

Equimolar amounts of **10** (0.01 mol) in ethanol and α-haloketones, namely chloroacetone (0.93 g, 0.01 mol) or chloroacetonitrile (0.76 g, 0.01 mol) or [chloroacetyl chloride (0.8 mL, 0.01 mol) or chloroacetic acid (0.95 g, 0.01 mol) or ethyl bromoacetate (1.11 mL, 0.01 mol)], were stirred for 6 h at room temperature, then left to stand at the same temperature for 24 h. The separated solid material was washed with water, dried and crystallized from ethanol to give **13**, **15** and **17**, respectively.

*3-(2-Oxopropylthio)-3-(phenylamino)-2-tosylacrylonitrile (**13**)*

White crystals, yield 94%, mp: 220°C, IR (KBr): ν_{max}, cm^{-1}: 3287 (NH), 2220 (CN), 1730 (CO), 1603 (Ph); ^1H NMR (DMSO-d_6): δ 1.9 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 3.7 (s, 2H, CH_2), 7.12–7.97 (m, 10H, Ar–H), 9.25 (s, 1H, NH). MS (*m/z*, %): 386 (M^+, 100.0), 386 (100.0), 321 (64.8), 231 (45.7), 143 (35.2), 86 (19.9). Anal. Calcd. for $C_{19}H_{18}N_2O_3S_2$ (386.49): C, 59.05; H, 4.69; N, 7.25%. Found: C, 59.11; H, 4.76; N, 7.31%.

*3-(Cyanomethylthio)-3-(phenylamino)-2-tosylacrylonitrile (**15**)*

White powder, yield 89%, mp: 270°C, IR (KBr): ν_{max}, cm^{-1}: 3200 (NH), 2220, 2195 (CN), 1603 (Ph); ^1H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 3.23 (s, 2H, CH_2), 7.12–7.87 (m, 9H, Ar), 9.25 (s, 1H, NH). MS (*m/z*, %): 367 (M^+−2, 1.4), 279 (50.0), 246 (40.3), 192 (20.8), 155 (40.3), 123 (50.0), 84 (100.0). Anal. Calcd. for $C_{18}H_{15}N_3O_2S_2$ (369.46): C, 58.52; H, 4.09; N, 11.37%. Found: C, 58.47; H, 4.03; N, 11.43%.

*Ethyl 2-(2-cyano-1-(phenylamino)-2-tosylvinylthio)acetate (**17**)*

White crystals, yield 87%, mp: 290°C, IR (KBr): ν_{max}, cm^{-1}: 3278 (NH), 2220 (CN), 1730 (CO); ^1H NMR (DMSO-d_6): δ 1.3 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 3.7 (s, 2H, CH_2), 4.3 (q, 2H, CH_2), 7.5–8.56 (m, 9H, Ar), 9.25 (s, 1H, NH). MS (*m/z*, %): 416 (M^+, 94.3), 278 (10.8), 246 (21.3), 155 (46.3), 123 (20.2), 84 (100.0). Anal. Calcd. for $C_{20}H_{20}N_2O_4S_2$ (416.51): C, 57.67; H, 4.84; N, 6.73%. Found: C, 57.74; H, 4.87; N, 6.77%.

*General procedure for the synthesis of thiophene derivatives **14**, **16** and **18***

Method A: A mixture of equimolar amounts of **10** (0.01 mol) and α-haloketones, namely chloroacetone (0.93 g, 0.01 mol) or chloroacetonitrile (0.76 g, 0.01 mol) or [chloroacetyl chloride (0.8 mL, 0.01 mol) or chloroacetic acid (0.95 g, 0.01 mol) or ethyl bromoacetate (1.11 mL, 0.01 mol)], was refluxed in DMF (20 mL) for 6 h. The reaction mixture was cooled, filtered off, dried and recrystallized from ethanol to give the corresponding thiophene derivatives **14**, **16** and **18**, respectively.

Method B: Refluxing of **13** (3.86 g, 0.01 mol) or **15** (3.69 g, 0.01 mol) or **17** (4.17 g, 0.01 mol) in ethanol (20 mL) containing a catalytic amount of TEA (0.5 mL) for 3 h afforded the corresponding substituted thiophene derivatives **14**, **16** and **18**, respectively.

*1-(3-Amino-5-(phenylamino)-4-tosylthiophen-2-yl)-ethanone (**14**)*

White powder, yield 88%, mp: 195°C, IR (KBr): ν_{max}, cm^{-1}: 3438 (NH_2), 3286 (NH), 1650 (CO), 1602 (Ph); ^1H NMR (DMSO-d_6): δ 1.9 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 7.2–7.9 (m, 9H, Ar), 8.2 (d, 2H, NH_2), 9.5 (s, 1H, NH). MS (*m/z*, %): 386 (M^+, 100.0), 386 (100.0), 321 (64.8), 231 (45.7), 143 (35.2), 86 (19.9). Anal. Calcd. for $C_{19}H_{18}N_2O_3S_2$ (386.49): C, 59.05; H, 4.69; N, 7.25%. Found: C, 59.01; H, 4.62; N, 7.19%.

*3-Amino-5-(phenylamino)-4-tosylthiophene-2-carbonitrile (**16**)*

Black powder, yield 80%, mp: 240°C, IR (KBr): ν_{max}, cm^{-1}: 3456 (NH_2), 3266 (NH), 2220 (CN), 1603 (Ph); ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.5 (br, 2H, NH_2), 7.31–7.96 (m, 9H, Ar–H), 8.7 (s, 1H, NH). MS (*m/z*, %): 367 (M^+, 1.4), 279 (50.0), 246 (40.3), 192 (20.8), 155 (40.3), 123 (50.0), 84 (100.0). Anal. Calcd. for $C_{18}H_{15}N_3O_2S_2$ (369.46): C, 58.52; H, 4.09; N, 11.37%. Found: C, 58.61; H, 4.13; N, 11.44%.

*Ethyl 3-amino-5-(phenylamino)-4-tosylthiophene-2-carboxylate (**18**)*

Black powder, yield 76%, mp: 240°C, IR (KBr): ν_{max}, cm^{-1}: 3459 (NH_2), 3301 (NH), 1743 (CO), 1598 (Ph); ^1H NMR (DMSO-d_6): δ 1.26 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 4.22 (q, 2H, CH_2), 6.55 (br, 2H, NH_2), 7.18–7.96 (m, 9H, Ar–H), 19.25 (s, 1H, NH). MS (*m/z*, %): 416 (M^+, 94.3), 278 (10.8), 246 (21.3), 155 (46.3), 123 (20.2), 84 (100.0). Anal. Calcd. for $C_{20}H_{20}N_2O_4S_2$ (416.51): C, 57.67; H, 4.84; N, 6.73%. Found: C, 57.74; H, 4.87; N, 6.75%.

*Synthesis of 4-amino-3-phenyl-5-tosylthiazole-2(3H)-thione (**19**)*

A mixture of equimolar amounts of **1** (1.95 g, 0.01 mol), elemental sulfur (0.32 g, 0.01 mol) and phenyl isothiocyanate (1.2 mL, 0.01 mol) in ethanol (10 mL) containing a catalytic amount of TEA (0.5 mL) was stirred overnight, then refluxed for 2 h. The reaction mixture was poured on ice-water, then filtered off, dried and recrystallized from ethanol to give the thiazole derivative **19**. Green powder, yield 90%, mp: 270°C, IR (KBr): ν_{max}, cm^{-1}: 3480, 3390 (NH_2); ^1H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 6.1 (d, 2H, NH_2), 7.2–8.1 (m, 9H, Ar-H). MS (*m/z*, %): 362 (M^+, 7.5), 321 (8.8), 291 (12.5), 274 (20.0), 194 (12.5), 167 (16.3), 135 (52.5), 77 (100.0). Anal. Calcd. for $C_{16}H_{14}N_2O_2S_3$ (362.49): C, 53.01; H, 3.89; N, 7.73%. Found: C, 52.92; H, 3.85; N, 7.69%.

Antioxidant screening assay (ABTS method) [20]

L-Ascorbic acid was obtained from Sigma, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was purchased from Wak and all other chemicals were of the highest quality available. For each of the investigated compounds, 2 mL of ABTS solution (60 μM) was added to 3 M magnesium oxide (MnO_2) solution (25 mg/mL) all prepared in phosphate buffer (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at λ 734 nm. Then, 50 μL of (2 mM) solution of the test compound in spectroscopic grade methanol/phosphate buffer (1:1) was added. The absorbance (A_{test}) was measured and the reduction in color intensity was expressed as % inhibition. The inhibition for each compound was calculated from the following equation.

$$\%\,\text{Inhibition} = [A(\text{control}) - A(\text{test})/A(\text{control})] \times 100$$

Ascorbic acid (vitamin C) was used as standard antioxidant (positive control). Blank sample was run without ABTS and using methanol/phosphate buffer (1:1) instead of sample. Negative control sample was run with methanol/phosphate buffer (1:1) instead of tested compound.

Antioxidant activity screening assay for erythrocyte haemolysis

The blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat was washed three times with 10 volumes of 0.15 M NaCl. During the last wash, the erythrocytes were centrifuged at 2500 rev./min for 10 min to obtain a constantly packed cell preparation. Erythrocyte haemolysis was mediated by peroxyl radicals in this assay system. A 10% suspension of erythrocytes in phosphate buffered saline pH 7.4 (PBS) was added to the same volume of 200 mM 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) solution (in PBS) containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37°C for 2 h. The reaction mixture was then removed, diluted with eight volumes of PBS and centrifuged at 1500 g for 10 min. The absorbance of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 volumes of distilled water to achieve complete haemolysis, and the absorbance of the supernatant obtained after centrifugation was measured at 540 nm. The data percentage haemolysis was expressed as mean-standard deviation. L-Ascorbic acid was used as a positive control.

Bleomycin-dependent DNA damage assay

The reaction mixtures contained, in a final volume of 1.0 mL, the following reagents at the final concentrations stated: DNA (0.2 mg/ mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM), magnesium chloride (5 mM), KH_2PO_4–KOH buffer pH 7.0 (30 mM) and ascorbic acid (0.24 Mm) or the compounds tested in MeOH to give a concentration of (0.1 mg/mL). The reaction mixtures were incubated in a water-bath at 37°C for 1 h. At the end of the incubation period, 0.1 mL of 0.1 M ethylenediaminetetraacetic acid (EDTA) was added to stop the reaction (the iron-EDTA complex is unreactive in the bleomycin assay). DNA damage was assessed by adding 1 mL 1% (w/v) thiobarbituric acid (TBA) and

1 mL 25% (v/v) hydrochloric acid (HCl) followed by heating in a water-bath maintained at 80°C for 15 min. The chromogen formed was extracted into butan-1-ol and the absorbance was measured at 532 nm [21, 22].

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