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SONOCHEMICAL SYNTHESIS OF 4-AMINOANTIPYRINE SCHIFF BASES AND EVALUATION OF THEIR ANTIMICROBIAL, ANTI-TYROSINASE AND DPPH SCAVENGING ACTIVITIES

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Abstract. A series of 4-(substituted benzylidene-amino)-2,3-dimethyl-1-phenyl-3-pyrazoline-5-ones (**3a-l**) was obtained using sonochemical assisted reaction. The synthesized Schiff bases were evaluated for their antiviral, antibacterial, anticandida, DPPH scavenging and anti-tyrosinase activities *in vitro*. The antimicrobial screening results revealed that amongst the tested imines, compound **3l** appeared to be a promising one (with a minimal inhibitory concentration 47 ug/ml). The most potent DPPH scavengers were azomethines 3**a** and 3**g**. No significant inhibition on the monophenolase activity *in vitro* of mushroom tyrosinase was observed.

Keywords: 4-aminoantipyrine; Schiff bases; mushroom tyrosinase; antimicrobial activity; DPPH scavenging activity

Introduction

4-Aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazoline-5-one) is an ubiquitous skeleton belonging to a five-membered partially saturated heterocyclic compounds with two adjacent nitrogen atoms. Antipyrine derivatives are of great worth due to the broad biological and pharmacological activities they exert: analgesic and anti-inflamatory (Turan-Zitouni et al., 2001; Sondhi et al., 2000; Yoshioka et al., 1981; Burdulene et al., 1999; Costa et al., 2006; Wong, 2002), antibacterial (Ali et al., 2012; Sayed et al., 1992; Vaghasiya et al., 2004), antiviral (Evstopov et al., 1992), antioxidant (Alam et al., 2012; Alam & Lee, 2012; Santos et al., 2010; Teng et al., 2011) activities. Besides their prominent biological significance, such compounds are also used as effective corrosion inhibitors (Govindaraju et al., 2009; Frignani et al., 1999; Huynh et al., 2000; Popova et al., 2004; Bentiss et al., 1999; Gopi et al., 2007).

Since the discovery of imines by Hugo Schiff as efficient metalloligands and later named in his honour, versatile condensation products bearing an imino (-N=CH–) group have been obtained. Schiff bases have also been known as anils, imines or azomethines which generally derived from the interaction of a carbonyl compound (aldehyde or ketone) and primary amine. Generally, the preferable methods for preparation of Schiff bases use aromatic aldehydes due to the formation of more effective conjugated systems in comparison with those, formed by condensation with aliphatic aldehydes.

It is noteworthy to mention that the optoelectronic properties of conjugated Schiff bases find application in organic electronics such as organic and perovskite solar cells (Hindson et al., 2010; Petrus et al., 2015).

Indeed, the outstanding coordination behavior endows the mentioned compounds to be important not only in the field of analytical chemistry (Jungreis & Thabet, 1969), but either in biochemistry.

Azomethines are common enzymatic intermediates of various biochemical conversions. Moreover, they are also found as potent inhibitors of various enzymes such as β-glucosidase, lipoxygenase, urease, etc. (Aslam et al., 2015; Raykova et al., 2017). In this context, Yu. et al. (2015) considered the Schiff base as effective tyrosinase inhibitors. Tyrosinase is known as an oxidase, comprises two distinct reactions of melanogenesis: hydroxylation of a monophenol and further the conversion of an *o*-diphenol to the corresponding *o*-quinone (Yaneva et al., 2017; Olivares & Solano, 2009; Robb, 1984). However, the tyrosinases isolated from different (plant, animal, and fungal) sources differ with respect to their primary structure, size and activation characteristics, and others. All those tyrosinases possess two copper atoms which are coordinately bonded with three histidine residues at the tyrosinase active site (Decker & Tuczek, 2000). Decreasing tyrosinase activity has recently gained the attention of researchers due to the potential application of the corresponding compounds in medicine, in cosmetics and in agriculture.

Literature survey reveals that Schiff bases are also highly efficient scavengers of various radical oxygen species (Alam et al., 2012). They inhibit the NO (nitric oxide) production, which is associated with effect on inflammatory mediator macrophages during the inflammation process (e.g., Diabetes type-1, arthritis) and human carcinogenesis (Surh et al., 2001).

Considering the significant chemical and biological importance of azomethines, the aim of the present work was to synthesize a serias of 4-aminoantipyrine derived Schiff bases via sonochemical reaction and to assess their *in vitro* antioxidant, antimicrobial and tyrosinase inhibitory activities.

Results

Sonochemical synthesis of the Schiff base analogues of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one

Following the outlined a nucleophilic addition (A_N) reaction on **Scheme 1**, a series of Schiff base (**3a-l**) derived from 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (**2**)) and different substituted benzene carbaldehydes (**1**) have been synthesized.

Considering the greenness of organic reactions (Shoaib et al., 2015), herein the reaction is accelerated by sonochemical activation. The chemical structures of the azomethine products have been confirmed by melting points, UV, ^{1}H-and ^{13}C-NMR, IR and mass spectral data. The results from the detailed structural characterization of the prepared Schiff bases are shown in the Experimental section.

The IR spectra of the newly formed imino group in all synthesized azomethines display a strong –C=N– stretching absorption band around 1609–1558 cm^{-1}. Meanwhile, a carbonyl-carrying five membered pyrazoline-5-one ring is observed at a similar strong absorption at 1658–1615 cm^{-1}.

The results from the analysis of the ^{1}H and ^{13}C NMR spectra unambigiosly confirmed the structure of the synthesized azomethines **3a-l**. The comlete assignment of the spectra is presented in the Experimental part. In the ^{1}H NMR spectra of all product it was registered singlet resonance signal at 9.36 – 9. 77 ppm characteristic for the imine proton of azomethines. The ^{13}C-NMR spectra of all products display resonance signal at 152.2-158.2 ppm characteristic for the –***CH***=N– skeletonof azomethines.

The positive-ion ESI-MS spectra of Schiff base (**3a–l**) consist of an intense protonated molecular ion peak and [M+Na]$^+$.

Biological activity: DPPH scavenging activity assessment

For estimation of primary index of antioxidant activity of Schiff bases, DPPH (1,1-diphenyl-2-picrylhydrazyl) test has been applied. This method is a rapid one and commercially available, stable, and cheap organic nitrogen free (1,1-diphenyl-2-picrylhydrazyl) radical is used. Amongst many variations of the spectrophotometric DPPH method, *Nenadis's* assay has been employed (Nenadis & Tsimidou, 2002).

DPPH scavenging abilities of the synthesized analogues (**3a–l**) of 4-aminoantipyrine has been carried out at a concentration of 48 μM along with the used standards (3,4-dihydroxybenzaldehyde and caffeic acid).

As shown on Fig. 1. the results of radical scavenging activities (% RSA) reveal that all tested azomethines show lower DPPH activities than the used positive controls.

As can be seen, **3l** is the least active compound (% RSA=2.5) with *para*-nitro substituent in its structure. Similarly, a poor activity is also found in compound **3h**, which possesses both a hydroxyl- and a deactivating nitro groups in 2, 5-position of benzylidene phenyl ring.

Comparison between azomethines (**3f** and **3k**) with a single hydroxyl group reveals that the *para*-substituted compound (**3f**) is more efficient as a scavenger than its *meta*-hydroxy analogue (**3k**). Moreover, introduction of an activating *N,N*-dimethylamino *para*-substituent (**3c**) leads to~2-fold increased activity. This observation is with agreement of previously reported in literature work (Alam et al., 2012).

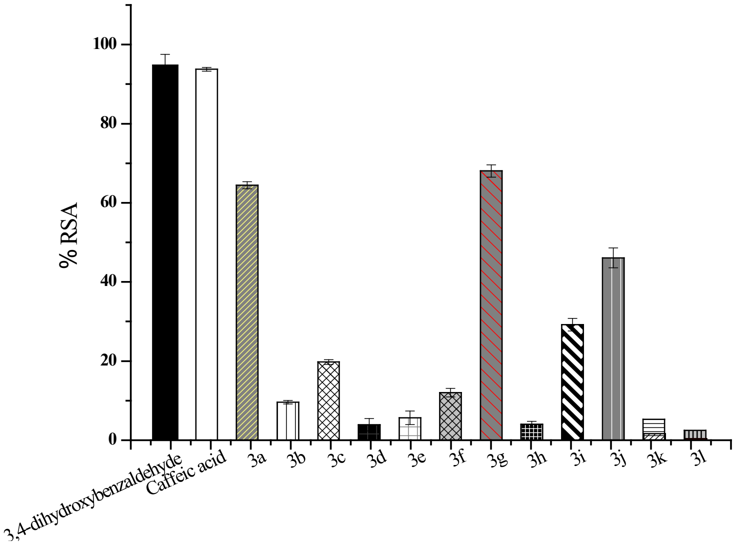


Figure 1. Radical scavenging activities (% RSA)

Azometines that have been born from 4-aminoantipyrine and vanillin or isovanillin (compounds **3a** or **3i**, respectively), demonstrated two folds greater scavenging activity at the expense of methoxy analogue with a *p*ara-phenolic hydroxyl group (**3a**).

Through the introduction into **3a** of second methoxy substituents *ortho* to *para*-hydroxyl group in benzylidene phenyl ring, leads to the most potent antioxidant compound **3g**. Our results also endorse the statement that the addition of one or two methoxy groups at the *ortho* position relative to the *para*-phenolic hydroxyl group increased DPPH-scavenging effect by stabilizing the aryloxyl radical (Cuvelier et al., 1992).

As expected, the significant decrease in radical-scavenging activities is due to the replacement of phenolic group/s of **3j** or **3g** with methoxy groups, which produced compounds **3d** and **3e**, respectively.

Surprisingly, although the presence of catechol moiety increases antioxidative activity, herein we have obtained that the DPPH scavenging activity of **3j** (3,4-dihydroxy benzylidene phenyl ring) has remained less efficient than compound **3g**. On the other hand, the presence of two hydroxyl phenolic groups in 2,4-position in benzylidene phenyl ring of azomethine (**3b**) leads to a significant decrease of the evaluated activity (up to 10 %).

In vitro antimicrobial activity

The development of novel antimicrobial drugs is an attractive area of research due to the increasing resistance of microorganisms to the currently available drugs. It is well known that various nosocomial clinical isolates, such as *Acinetobacter*, *Staphylococcus* and *Pseudomonas* display resistance to many of the commercial available antibiotics (Bonapace et al., 2000; Chait et al., 2007; Drago et al., 2004).

The reports in the literature about the antimicrobial activities of 4-aminoantipyrine core (Windholz & Budavari, 1983), along with the azomethine skeleton (Vaghasiya et al., 2004; Shi et al., 2007; da Silva et al., 2011; Mohini et al., 2013; Malladi et al., 2013; Shoaib et al., 2015; Kumar et al., 2017; Ei ashry et al., 2007) prompted us to obtain potential antimicrobials based on Schiff bases derived from 4-aminoantipyrine.

Table 1. Antibacterial and antifungal activities of Schiff bases expressed as minimal inhibitory concentrations MIC (µg.ml^{-1})

	MIC (µg.ml^{-1})		
Compound	*Staphylococcus aureus 209*	*Escherichia coli WF+*	*Candida albicans 562*
(3a)	NT	NT	NT
(3b)	1500	1500	0

(3c)	1500	0	375
(3d)	NT	NT	NT
(3e)	1500	0	0
(3f)	750	0	375
(3g)	750	750	375
(3h)	1500	0	375
(3i)	0	0	0
(3j)	1500*	0	375
(3k)	1500	1500	375
(3l)	47	750	375
Tobramicyn	15.6	19.5	N.T
Ketocon-azole	N.T	N.T	<7.8

The azomethines were evaluated for *in vitro* antibacterial activity against Gram-positive (*Staphylococcus aureus)* and Gram-negative (*Escherichia coli)* bacteria, whereas the antifungal activity was tested against *Candida albicans*. As a conventional antibacterial agent tobramycin and an antifungal agent ketoconazole were included in the assay for comparison.

The test results, presented in Table 1, disclose that most of the Schiff bases were active against tested pathogenic fungus, exhibiting an MIC value of 375 µg.ml^{-1}.

Moreover, *in vitro* antibacterial activity results indicate that among the tested azometines, compound **3l** with a *para*-nitro group in its skeleton show the most potent activity against *Staphylococcus aureus* 209, on the contrary to its the worst antioxidant activity. In respect to the antibiotic-tobramicyn, compound **3l** was 3-fold less active than the used reference drug.

The other Schiff bases have shown no expressive antibacterial activities against the both pathogenic bacterial strains.

In addition, the evaluation of antiviral activity of azometines (3a-l) against poliovirus type 1 (LSc-2ab) and coxsackievirus B1 (CV-B1), representing the Enterovirus genus of the *Picornaviridae* was also tested *in vitro*. None of the the tested Schiff bases showed antiviral effect against the replication of the representatives of the *Picornaviridae* family.

*In vitro effects of the Schiff bases (**3a-l**) on mushroom tyrosinase*

Pursuing the idea of finding of more potent and non-toxic tyrosinase inhibitors, herein we have estimated the synthetically obtained Schiff bases. Up to the best of our knowledge, this is the first report on evaluation of anti-tyrosinase activity of azomethines derived from 4-aminoantipyrine. Owing to the coordinating properties of Schiff base ligands, we assumed that the tested imines could be promising tyrosinase inhibitors, due to their abilities to form stable complexes with copper ions, which exist in the tyrosinase active center. In this study, we screened the anti-tyrosinase activity of synthesized compounds (3a-l) using a modified method from Kwak et al. (2011). *L*-Tyrosine was used as a monophenolase substrate of tyrosinase, and for comparison in the assay hydroquinone and kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone)) were selected as comparing standard tyrosinase inhibitors.

Nevertheless, the tested Schiff bases (**3a-l**) are well known as excellent metal ligands, our results (data not shown) have shown that the compounds have either poor or no inhibitory activity on mashroom tyrosinase. Meantime, these results suggested that our compounds can be considered rather as activators of tyrosinase.

Conclusions

In summary, 4-aminoantipyrine derived Schiff bases (**3a-l**) were prepared sonochemically. The synthesized compounds were determined by UV, IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS. The results of antioxidant activity revealed that amongst the Schiff bases tested, 4-aminoantipyrine analogues **3a** and **3g** (derived from vanillin and syringaldehyde, respectively) were the strongest scavengers of DPPH. Moreover, compound **3l** showed promising antibacterial activity with MIC value of 47 μg.ml^{-1}. Preliminary results on monophenolase activity of mushroom tyrosinase indicated that Schiff bases behaved as activators rather than inhibitors of the enzyme.

APPENDIX: EXPERIMENTAL

General

All chemicals (reagent grade) used were purchased from Sigma Aldrich (FOT, Bulgaria). Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60F$_{254}$ plates (Merck, Germany). The organic solvents were of analytical grade.

Melting points (mp) were determined on a Stuart SMP 10 apparatus, and the results were uncorrected. The UV spectra of the amides were measured with an Agilent 8453 UV–Vis spectrophotometer. Attenuated total reflectance infrared spectroscopy (ATR-IR) measurements were performed using Thermo Scientific Nicolet iS10 FT-IR device with ID5 ATR accessory (diamond crystal). The ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker Avance III 400 spectrometer, operating at 400.15 MHz for protons and 100.62 MHz for carbons. The spectra were recorded at a temperature of 25°C and spectral width of 5000 Hz and 20000 Hz for 1H and 13C, respectively. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane, and coupling constants (*J*) are reported in Hz.

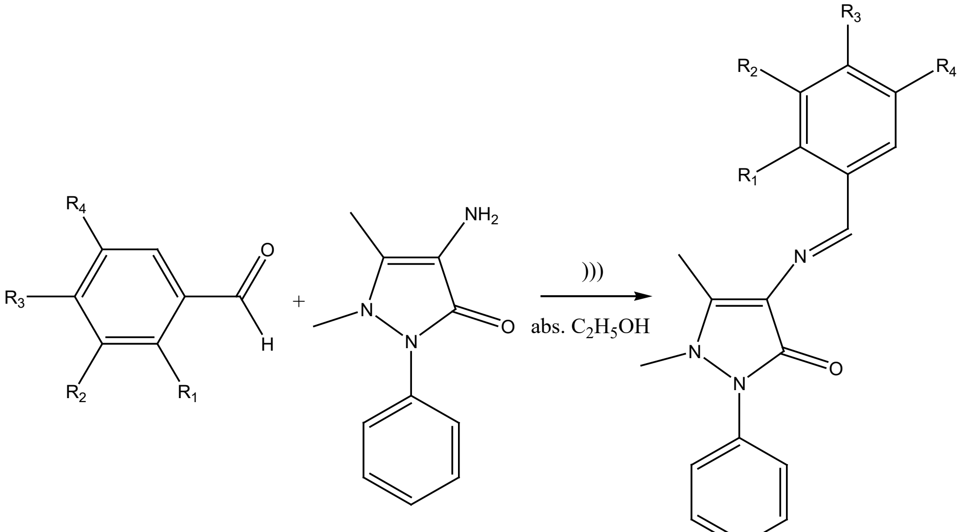
The mass spectra were obtained using an Esquire 3000 instrument (ESI pos. mode).-

*General method for the preparation of Schiff base analogues of 4-amino-2 3-dimethyl-1-phenyl-3-pyrazolin-5-one (**3a–l**)*

To 203.2 mg (1 mmol) 4-amino-2, 3-dimethyl-1-phenyl-3-pyrazoline-5-one dissolved in an anhydrous ethanol solution (10 mL) was added an anhydrous ethanol solution (10 mL) of substituted benzaldehyde (1 mmol), and the mixture was sonicated for 1–4 h under atmospheric conditions (Scheme 1). The progress of the reaction was monitored by TLC in PE:EtOAc/ HE:EtOAc. The obtained precipitate was filtered off and purified by recrystallization with ethanol, and then dried *in vacuo* to produce the pure compound.

*2,3–Dimethyl-1-phenyl-4-(4’-hydroxy-3’-methoxy benzylideneamino)-pyrazoline-5-one (**3a**)*

Lemon yellow crystal, yield 61%; M.p. 205-207°C; UV–Vis (C_2H_5OH, λ_{max} nm): 205, 233, 337 nm; ^{1}H NMR (DMSO-d_6, δ ppm *J* Hz): 2.44 (s, 3H, -$CC\underline{H}_3$), 3.16 (s, 3H, -$NC\underline{H}_3$), 3.82 (s, 3H, -OCH_3), 6.87 (d, 1H, J=8.1 Hz, Ar-H), 7.18 (d, 1H, J=2.2 Hz, Ar-H), 7.21 (dd, 1H, J=8.1, 2.2 Hz, Ar-H), 7.34 -7.39 (m, 2H, Ar-H), 7.51-7.55 (m, 3H, Ar-H), 9.47 (s, 1H, -N=CH-Ph); [IR (ATR)u_{max}] 3065, 2964, 1624, 1581, 1518, 1381, 1377, 1287, 1250, 1184, 1113, 1025, 768, 709 cm^{-1}; ESI-MS: m/z 338.2 $[M+H]^+$, 360.2 $[M+ Na]^+$.



1 **2** **3 a-l**

No	R_1	R_2	R_3	R_4	Yields, %
3a	H	OCH_3	OH	H	61
3b	OH	H	OH	H	90
3c	H	H	$N(CH_3)_2$	H	87
3d	H	OCH_3	OCH_3	H	72
3e	H	OCH_3	OCH_3	OCH_3	69
3f	H	H	OH	H	50
3g	H	OCH_3	OH	OCH_3	30
3h	OH	H	H	NO_2	81
3i	H	OH	OCH_3	H	89
3j	H	OH	OH	H	79
3k	H	OH	H	H	86
3l	H	H	NO_2	H	84

Scheme 1

*2,3–Dimethyl-1-phenyl-4-(2',4'-dihydroxy benzylideneamino)-pyrazoline-5-one (**3b**)*

Lemon yellow crystal, yield 90 %; M.p. 231-232 °C; UV–Vis (C_2H_5OH, λ_{max} nm): 207, 246, 347 nm; ^1H NMR (DMSO-d_6, δ ppm *J* Hz): 2.35 (s, 3H, -$C\underline{C}\underline{H}_3$), 3.14 (s, 3H, -$NC\underline{H}_3$), 6.25 (d, J=2.3 Hz, 1H, m-CH), 6.34 (dd, J= 8.4, 2.3 Hz, 1H, m-CH), 7.23 (d, J=8.4 Hz,1H, o-CH), 7.37 (m, 3H, o-+ p-CH), 7.52 (t, J= 7.8 Hz,1H, m-CH), 9.56 (s, 1H, -N=C$\underline{H}$-Ph), 10.10 (s,1H, OH), 13.31 (s, 1H, OH); ^{13}C NMR (DMSO-d_6, δ ppm): δ 9.8 (-$C\underline{C}H_3$), 35.4 (-$N\underline{C}H_3$), 102.4 (m-CH), 107.6 (m-CH), 124.5 (2 x o-CH, Ph), 127.0 (p-CH,Ph), 129.1 (2 x m-CH,Ph), 133.0 (o-CH), 134.4 (Cq), 149.6 (Cq, $N\underline{C}CH_3$), 158.2 (-N=CH), 159.4 (>CO), 161.2 (=COH), 161.8 (=COH); [IR (ATR)u_{max}] 3459, 3054, 1625, 1587, 1514, 1428, 1368, 1312, 1222, 1160, 1123, 834, 699 cm^{-1}; ESI-MS: m/z 324.3 [M+ H]$^+$, 346.2 [M+ Na]$^+$, 669.2 [2M+ Na]$^+$.

*2,3–Dimethyl-1-phenyl-4-(4’-dimethylamino) benzylideneamino)-pyrazoline-5-one (**3c**)*

Lemon yellow crystal, yield 87 %; M.p. 220-222°C; UV–Vis (C_2H_5OH, λ_{max} nm): 205, 237, 336 nm; ^{1}H NMR (DMSO-d$_6$, δ ppm *J* Hz): 2.51 (s, 3H,-CCH$_3$), 3.05 (s, 6H, –N(CH$_3$)$_2$), 3.15 (s, 3H, -N–CH$_3$), 6.79 (d, 2H, J = 8.2 Hz, Ar-H), 7.36–7.54 (m, 5H, Ar-H), 7.81 (d, 2H, J = 8.2 Hz, Ar-H), 9.73 (s, 1H, -N=CH-Ph); [IR (ATR)u$_{max}$] 1644, 1609, 1580, 1526, 1370, 1134, 816, 774 cm^{-1}; ESI-MS: m/z 335.3 [M+H]$^+$, 357.2 [M+Na]$^+$.

*2,3–dimethyl-1-phenyl-4-(3’,4’-dimethoxy benzylideneamino)-pyrazoline-5-one (**3d**)*

Lemon yellow crystal, yield 75 %; M.p. -191-192°C; UV–Vis (C_2H_5OH, λ_{max} nm): 204, 232, 336 nm; [IR (ATR)u$_{max}$] 1645, 1593, 1573, 1509, 1487,1414, 1257, 1276, 1136, 1017, 809, 768, 706 cm^{-1}; ^{1}H NMR (DMSO-d$_6$, δ ppm *J* Hz): 2.49 (s, 3H, -CCH$_3$), 3.12 (s, 3H, -N–CH$_3$), 3.91 (s, 3H, OCH$_3$), 3.94 (s, 3H, OCH$_3$), 6.89 (d, 1H, J = 2.4 Hz, Ar-H), 7.29–7.57 (m, 7H, Ar-H), 9.72 (s, 1H, -N=CH-Ph); ESI-MS: m/z 352.2 [M+H]$^+$, 374.2 [M+ Na]$^+$.

*2,3–dimethyl-1-phenyl-4-(3’,4’, 5’-trimethoxy benzylideneamino)-pyrazoline-5-one (**3e**)*

Lemon yellow crystal, yield 69%; M.p. 201-203°C; UV–Vis (C_2H_5OH, λ_{max} nm): 205, 233, 334 nm; ^{1}H NMR (DMSO-d$_6$, δ ppm *J* Hz): 2.46 (s, 3H, -CC$\underline{H}_3$), 3.20 (s, 3H, -NC$\underline{H}_3$), 3.71 (s, 3H, OCH$_3$), 3.84 (s, 6H, 2 x OCH$_3$), 7.14 (s, 2H, Ar-H), 7.36-7.55 (m, 5H, Ar-H), 9.61 (s, 1H, -N=C$\underline{H}$-Ph); ^{13}C NMR (DMSO-d$_6$, δ ppm): δ 9.9 (-C$\underline{C}$H$_3$), 35.4 (-N$\underline{C}$H$_3$), 55.8 (3 x O$\underline{C}$H$_3$), 104.8 (2 x *o*-CH), 116.8 (Cq), 124.0 (2 x o-CH, Ph), 127.3 (*p*-CH, Ph), 129.6 (2 x *m*-CH, Ph), 133.6 (Cq), 135.1 (Cq), 139.8 (Cq), 152.6 (Cq, =$\underline{C}$CH$_3$), 153.6 (=$\underline{C}$OCH$_3$), 154.7 (-N=CH-Ph), 160.1(>C=O); [IR (ATR)u$_{max}$] 1648, 1578, 1502, 1489, 1467, 1450, 1328, 1286, 1225, 1001, 951, 826, 700 cm^{-1}; ESI-MS: m/z 382.3 [M+ H]$^+$, 404.3 [M+ Na]$^+$.

*2,3–Dimethyl-1-phenyl-4-(4’-hydroxy benzylideneamino)-pyrazoline-5-one (**3f**)*

Lemon yellow crystal, yield 50 %; M.p. 233-234°C; UV–Vis (C_2H_5OH, λ_{max} nm): 207, 230, 331 nm; ^{1}H NMR (DMSO-d$_6$, δ ppm *J* Hz): 2.42 (s, 3H, -CC$\underline{H}_3$), 3.15 (s, 3H, -NC$\underline{H}_3$), 6.85 (d, 2H, J = 8.1 Hz, Ar-H), 7.36-7.54 (m, 5H, Ar-H), 7.67 (d, 2H, J = 2.5 Hz, Ar-H), 9.51 (s, 1H, -N=C$\underline{H}$-Ph); [IR (ATR)u$_{max}$] 3448, 2929, 1654, 1609, 1580, 1509, 1495, 1454, 1377, 1274, 1230, 1139, 761 cm^{-1}; ESI-MS: m/z 308.2 [M+ H]$^{+}$, 330.2 [M+ Na]$^{+}$.

*2,3–dimethyl-1-phenyl-4-(3',5'-dimethoxy-4'-hydroxy benzylideneamino)-pyrazoline-5-one (**3g**)*

Yellow crystal, yield 51 %; M.p. 258-261°C; UV–Vis (C_2H_5OH, λ_{max} nm): 205, 238, 339 nm; ^1H NMR (DMSO-d_6, δ ppm *J* Hz): 2.46 (s, 3H, -CCH_3), 3.12 (s, 3H, –NCH_3), 3.90 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.97 (d, 1H, J = 2.4 Hz, Ar-H), 7.30–7.52 (m, 5H, Ar-H), 9.49 (s, 1H, -N=CH-Ph); [IR (ATR)u_{max}] 3158, 2995, 1632, 1587, 1507, 1466, 1456, 1352, 1293, 1251,1208, 1141, 1111, 965, 771 cm^{-1}; ESI-MS: m/z 368.2 [M+ H]$^{+}$, 390.2 [M+ Na]$^{+}$.

*2,3–dimethyl-1-phenyl-4-(5'-Nitro-2'-hydroxy benzylideneamino)-pyrazoline-5-one (**3h**)*

Yellow crystal, yield 68 %; M.p. 235-236°C; UV–Vis (C_2H_5OH, λ_{max} nm): 206, 308, 352 nm; ^1H NMR (DMSO-d_6, d ppm J Hz): δ 2.43 (s, 3H, -$CC\underline{H}_3$), 3.24 (s, 3H, -$NC\underline{H}_3$), 7.07 (d, J=8.9 Hz, 1H, m-CH), 7.38 (d, J= 8.3 Hz, 2H, o-CH,Ph), 7.41 (t, J=7.4 Hz,1H, p-CH, Ph), 7.54 (dd, J=8.3,7.4 Hz, 2H, m-CH, Ph), 8.15 (dd, J= 8.9, 2.8 Hz,1H, p-CH), 8.52 (d, J=2.8 Hz, 1H, o-CH), 9.77 (s, 1H, -N=C$\underline{H}$-Ph), 13.75 (s, 1H, OH); ^{13}C NMR (DMSO-d_6, δ ppm): δ 9.8 (-$C\underline{C}H_3$), 34.9 (-$N\underline{C}H_3$), 117.4 (m-CH), 125.2 (2 x o-CH, Ph), 125.4 (o-CH), 126.7 (p-CH), 127.5 (p-CH,Ph), 129.2 (2 x m-CH,Ph), 134.0 (Cq), 139.7 (Cq), 150.6 (Cq, $N\underline{C}CH_3$), 153.3 (-N=CH), 158.8 (>CO), 164.5 (=COH); [IR (ATR)u_{max}] 3081, 1652, 1622, 1558, 1517, 1433, 1332, 1289, 1232, 1070, 751, 699 cm^{-1}; ESI-MS: m/z 353.3 [M+H]$^{+}$, 375.2 [M+Na]$^{+}$, 727.2 [2M+Na]$^{+}$.

*2,3–Dimethyl-1-phenyl-4-(3'-hydroxy-4'-methoxy benzylideneamino)-pyrazoline-5-one (**3i**)*

Beige powder, yield 89 %; M.p. 245-247°C; UV–Vis (C_2H_5OH, λ_{max} nm): 206, 231, 335 nm; ^1H NMR (DMSO-d_6, δ ppm J Hz): δ 2.42(s, 3H, -$CC\underline{H}_3$), 3.18 (s, 3H, -$NC\underline{H}_3$), 3.89 (s, 3H, OCH3), 6.78 (d, 1H, J=8.1 Hz, Ar-H), 7.01 (dd, 1H, J = 8.1, 1.8 Hz, Ar-H), 7.15-7.58 (m, 6H, Ar-H), 9.45 (s, 1H, -N=C$\underline{H}$-Ph); [IR (ATR) u_{max}] 3065, 2999, 2965, 1608, 1594, 1585, 1518, 1308, 1287, 1250, 1184, 1113, 1026, 768, 710 cm^{-1}; ESI-MS: m/z 338.2 [M+H]$^{+}$, 360.2 [M+Na]$^{+}$.

*2,3–dimethyl-1-phenyl-4-(3',4'-dihydroxy benzylideneamino)-pyrazoline-5-one (**3j**)*

Yellow crystal, yield 68%; M.p. 287-289°C; UV–Vis (C_2H_5OH, λ_{max} nm): 206, 234, 337 nm; ^1H NMR (DMSO-d_6, δ ppm *J* Hz): δ 2.39 (s, 3H, -$CC\underline{H}_3$), 3.11 (s, 3H, $-NC\underline{H}_3$), 6.77 (d, 1H, J=7.9 Hz, m-CH), 7.01 (dd, 1H, J= 7.9, 2.0 Hz, o-CH), 7.28 (d, 1H, J=2.0 Hz, o-CH), 7.35 (m, 3H, o-+ p-CH), 7.50 (t, 1H, J= 7.9 Hz, m-CH), 9.36 (s, 1H, $-N=C\underline{H}$-Ph); ^{13}C NMR (DMSO-d_6, δ ppm): δ 9.8 ($-C\underline{C}H_3$), 35.6 ($-N\underline{C}H_3$), 113.1 (o-CH), 115.6 (m-CH), 117.1 (Cq), 120.9 (o-CH), 124.4 (o-CH, Ph), 126.7 (p-CH, Ph), 129.2 (m-CH, Ph), 134.8 (Cq), 145.6 (=COH), 148.2 (=COH), 151.6 (Cq, $N\underline{C}CH_3$), 155.3 (-N=CH-Ph), 160.0 (-N-CO); [IR (ATR)u_{max}] 3490, 1617, 1583, 1526, 1487, 1446, 1378, 1388, 1263, 865, 769, 705 cm^{-1}; ESI-MS: m/z 324.3 $[M+H]^+$, 346.2 $[M+Na]^+$, 669.2 $[2M+Na]^+$.

*2,3–Dimethyl-1-phenyl-4-(3’-hydroxy benzylideneamino)-pyrazoline-5-one (**3k**)*

Beidge solid, yield 86 %; M.p. 266-270°C; UV–Vis (C_2H_5OH, λ_{max} nm): 206, 234, 337 nm; ^{1}H NMR (DMSO-d_6, δ ppm *J* Hz): δ 2.44 (s, 3H, $-CC\underline{H}_3$), 3.17 (s, 3H, $-NC\underline{H}_3$), 7.27-7.36 (m, 5H, Ar-H), 7.46-7.48 (m, 3H, Ar-H), 7.61-7.64 (t, 1H, m-CH), 9.58 (s, 1H, $-N=C\underline{H}$-Ph), 9.65 (s, 1H, OH); ^{13}C NMR (DMSO-d_6, δ ppm): δ 9.7 ($-C\underline{C}H_3$), 35.5 ($-N\underline{C}H_3$), 112.8 (o-CH), 116.3 (m-CH), 117.5 (Cq), 118.9 (o-CH), 124.6 (2 x o-CH, Ar), 126.9 (p-CH, Ph), 129.1 (2 x m-CH, Ph), 134.6 (Cq), 138.9 (Cq), 151.9 (Cq, $N\underline{C}CH_3$), 154.4 (-N=CH-Ph), 157.5 ($-N-\underline{C}O$), 159.6 (=COH); [IR (ATR)u_{max}] 3139, 1615, 1591, 1578, 1449, 1387, 1285, 1235, 765, 706, 688 cm^{-1}; ESI-MS: m/z 308.2 $[M+ H]^+$, 330.2 $[M+ Na]^+$.

*2,3–Dimethyl-1-phenyl-4-(4’-nitro benzylideneamino)-pyrazoline-5-one (**3l**)*

Orange solid, yield 86 %; M.p. -252-254°C; UV–Vis (C_2H_5OH, λ_{max} nm): 205, 248, 277, 384 nm; ^{1}H NMR (DMSO-d_6, δ ppm J Hz): δ 2.47 (s, 3H, $-CC\underline{H}_3$), 3.25 (s, 3H, $-NC\underline{H}_3$), 7.37-7.56 (m, 5H, Ar-H), 8.06 (d, 2H, Ar-H), 8.28 (d, 2H, Ar-H), 9.66 (s, 1H, $-N=C\underline{H}$-Ph); ^{13}C NMR (DMSO-d_6, δ ppm): δ 9.7($-C\underline{C}H_3$), 34.9 ($-N\underline{C}H_3$), 111.3 (*m*-CH, Ar), 115.6 (*m*-CH, Ar), 124.1 (2 x *o*-CH, Ph), 125.3 (2 x *o*-CH, Ar), 127.4 (*p*-CH, Ar), 127.9 (2 x CH, Ar), 129.2 (2 x *m*-CH, Ph), 134.2 (Cq), 143.6 (Cq), 147.8 (Cq), 150.8 (Cq, $N\underline{C}CH_3$), 152.2 (-N=CH), 159.1 (>CO); [IR (ATR) u_{max}] 3103, 3078, 2960, 1658, 1615, 1571, 1551, 1514 , 1336, 1313, 1129, 852, 763, 740, 689 cm^{-1}; ESI-MS: m/z 337.2 $[M+H]^+$, 359.2 $[M+ Na]^+$.

Evaluation of DPPH free radical scavenging activity (RSA)

Each compound and concentration tested (3.6 mM) was mixed with 0.1 mM ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH$^\bullet$) stable free radical. The absorption at 516 nm was measured at the start and at 10 and 20 min. The tests were performed in triplicate at 27ºC and the results are expressed as percent radical scavenging activity: % RSA = [Abs$_{516\ \mathrm{nm}\ (t = 0)}$) -Abs$_{516\ \mathrm{nm}\ (t = t0)}$ x 100/Abs$_{516\ \mathrm{nm}\ (t = 0)}$], as proposed by *Nenadis N.et al.* (Nenadis & Tsimidou, 2002).

In vitro antibacterial and antifungal activities

The *in vitro* antibacterial activities of synthesized azometines were screened against the *Staphylococcus aureus 209* (G+), Escherichia coli *WF*+ (G-), whereas the antifungal activities were tested against pathogenic fungus *Candida albicans* 562. All microorganisms were obtained from the Bulgarian National Collection for Microorganisms and Cell Cultures (NBIMCC).

The minimal inhibitory concentration (MIC) of all samples was determined by the microdilution method described by Andrews (Andrews, 2001). Briefly, 50 μl of twofold serial dilutions of examined samples were added to 50 μl microbial suspension adjusted to yield approximately 1.0×10^5 CFU ml^{-1}. MIC was encountered as the lowest concentration of examined sample that inhibits the visible microbial growth after 24 h incubation at 37°C. For positive controls commercially available antibiotic tobramycin and ketoconazole were used. The solvent DMSO was tested as negative controls. Three replicates were done for each compound.

Antiviral tests

The antiviral activity of the newly synthesized compounds was tested by the multicycle viral cytopathic effect (CPE) inhibition assay. Monolayer FL cells in 96-well plates were inoculated with 0.1 mL of the corresponding virus suspension containing 100 CCID$_{50}$. Mock-infected cells served as toxicity and cell controls. After an hour for virus adsorption at 37°C virus inocula was discarded and the so treated cells were further inoculated with 0.2 mL of maintenance medium containing 0.5 L/g concentrations of the compounds tested. Cells were incubated in a humidified atmosphere at 37°C and 5% CO$_2$. When virus cytopathic effect (CPE) reached its maximum (on the 48th hour p.i.), viable cells were stained according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] procedure with slight modifications and the optical density (OD) of each well was read with a spectrophotometer at 540 nm, with 690 nm as a reference read-out. The percentage of CPE inhibition was calculated according to the following formula: % CPE = [OD$_{\text{test sample}}$ – OD$_{\text{virus control}}$] / [OD$_{\text{toxicity control}}$ – OD$_{\text{virus control}}$] x 100 (Sudo et al., 1994); Pannecouque et al., 2008).

Cells and viruses

The experiments for testing the antienteroviral activity of the synthesized compounds were carried out with poliovirus type 1 [PV-1 (LSc-2ab)] and coxsackievirus B1 (CV–B1) from the collection of The Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences, Sofia, Bulgaria. Cells were grown in a humidified atmosphere at 37ºC and 5% CO_2 in a growth medium of Dulbecco modified Eagle's medium (DMEM) (Gibco®, Invitrogen), containing 5% fetal bovine serum (Gibco®, Invitrogen), supplemented with antibiotics (penicillin 100 IU/mL, streptomycin 100 µg/mL and gentamycin 50 µg/mL) and 20 mM HEPES buffer (Gibco®, Invitrogen). For the antiviral assays serum in the maintenance medium was reduced to 0.5%.

Mushroom tyrosinase activity assay

Tyrosinase activity assay was performed as previously described modified dopachrome method (Kwak et al., 2011) using *L*-tyrosine as the substrate (Chochkova et al., 2014).

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REFERENCES

Alam, M.S. & Lee, D.-U. (2012). Synthesis, molecular structure and antioxidant activity of (E)-4-[benzylideneamino]-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one, a Schiff base ligand of 4-aminoantipyrine. *J. Chem. Crystallogr., 42*, 93 – 102.

Alam, M.S., Choi, J.-H. & Lee, D.-U. (2012). Synthesis of novel Schiff base analogues of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one and their evaluation for antioxidant and anti-inflammatory activity. *Bioorg. Med. Chem., 20*, 4103 – 4108.

Ali, P., Meshram, J.S., Sheikh, J.I., Tiwari, V., Dongre, R.S. & ben Hadda, T. (2012). Predictions and correlations of structure activity relationship of some aminoanthypirine derivatives on the basis of theoretical and experimental ground. *Med. Chem. Res., 21*, 157 – 164.

Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemotherapy, 48*(S1), 5 – 16 .

Aslam, M., Anis, I., Noreen, Z., Hussain, A., Iqbal, L., Hussain, A., Iqbal, L., Hussain, A., Kumar, N. & Iqbal, S. (2015). Potent enzyme inhibitors: synthesis and spectral analysis of some new Schiff bases having nucleus of azomethine and screened their biological evaluation. *Int. J. Pharm. Sci. Rev. & Res., 32*, 148 – 152.

Bentiss, F., Lagrenee, M., Traisnel, M. & Hornez J.C. (1999). The corrosion inhibition of mild steel in acidic media by a new triazole derivative. *Corros. Sci., 41*, 789 – 803.

Bonapace, C.R., White, R.L., Friedrich, L.V. & Bosso, J.A. (2000). Evaluation of antibiotic synergy against acinetobacter baumannii: a comparison with Etest, time-kill, and checkerboard methods. *Diagn. Microbiol. Infect. Dis. , 38*, 43 – 50.

Burdulene, D., Palaima, A., Stumbryavichyute, Z. & Talaikite, Z.A. (1999). Synthesis and antiinflammatory activity of 4-aminoantipyrine derivatives of succinamides. *Pharm. Chem. J., 33*, 191 – 193.

Chait, R., Craney, A. & Kishony, R. (2007). Antibiotic interactions that select against resistance. *Nature, 446*, 668 – 671.

Chochkova, M.G., Georgieva, A.P., Ivanova, G.I., Nikolova, N., Mukova, L., Nikolaeva-Glomb, L. & Milkova, T.S. (2014). Synthesis and biological activity of hydroxycinnamoyl containing antiviral drugs. *J. Serb. Chem. Soc. 79*, 517 – 526 .

Costa, D., Marques, A.P., Reis, R.L., Lima, J.L. & Fernandes, E. . (2006). Inhibition of human neutrophil oxidative burst by pyrazolone derivatives. *Free Radical Biol. Med., 40*, 632 – 640.

Cuvelier, M.-E., Richard, H. & Berset, C. (1992). Comparison of the antioxidative activity of some acid-phenols: structure-activity relationship. *Biosci. Biotechnol. & Biochem., 56*, 324 – 325.

Da Silva, C.M., da Silva, D.L., Modolo, L.V., Alves, R.B., de Resende, M.A., Martins, C.V. B. & de Fátima, A. (2011). Schiff bases: a short review of their antimicrobial activities. *J. Adv. Res., 2*, 1 – 8.

Decker, H. & Tuczek, F. (2000). Tyrosinase/catecholoxidase activity of hemocyanins: structural basis and molecular mechanism. *Trends Biochem. Sci., 25*, 392 – 397.

Drago, L., De Vecchi, E., Nicola, L., Colombo, A., Guerra, A. & Gismondo, M.R. (2004). Activity of levofloxacin and ciprofloxacin in combination with cefepime, ceftazidime, imipenem, piperacillin-tazobactam and amikacin against difference Pseudomonas aeruginosa phenotypes and Acinetobacter spp. *Chemotherapy, 50*, 202 – 210.

Ei ashry, E.S.H., Awad, L.F., Ibrahim, E.I. & Bdeewy, O.K. (2007). Synthesis of antipyrine derivatives derived from dimedone. *Chinese J. Chem., 25*, 570 – 573 .

Evstopov, A.N., Yavorovskaya, V.E., Vorobev, E.S.,Khudonogova, Z.P., Gritsenko, L.N., Schmidt, E.N.,Medvedeva, S.G., Filimonov, D.V., Prishchep, T.P. & Saratikov, A.S. (1992). Synthesis and antiviral activity of antipyrine derivatives. *Pharm. Chem. J., 26*, 426– 430.

Frignani, A., Tommesani, L., Brunoro, G., Monticelli, C. & Fogagnolo, M. (1999). Influence of the alkyl chain on the protective effects of 1,2,3-benzotriazole towards copper corrosion.: part I: inhibition of the anodic and cathodic reactions. *Corros. Sci., 41*, 1205 – 1215.

Gopi, D., Govindaraju, K.M., Manimozhi, S., Ramesh, S. & Rajeswari, S. (2007). Inhibitors with biocidal functionalities to mitigate corrosion on mild steel in natural aqueous environment. *J. Appl. Electrochem., 37*, 681 – 689.

Govindaraju, K.M., Gopi, D. & Kavitha L. (2009). Inhibiting effects of 4-amino-antipyrine based schiff base derivatives on the corrosion of mild steel in hydrochloric acid. *J. Appl. Electrochem, 39*, 2345 – 2352.

Hindson, J.C., Ulgut, B., Friend, R.H., Greenham, N.C., Norder, B., Kotlewski, A. & Dingemans, T.J. (2010). All-aromatic liquid crystal triphenylamine-based poly(azomethine)s as hole transport materials for opto-electronic applications. *J. Mater. Chem., 20*, 937 – 944.

Huynh, N., Bottle, S.E., Notoya, T. & Schweinsberg, D.P. (2000). Inhibitive action of the octyl esters of 4- and 5-carboxybenzotriazole for copper corrosion in sulphate solutions. *Corros. Sci., 42*, 259 – 274.

Jungreis, E. & Thabet, S. (1969). Analytical application of schiff bases (pp. 149-177). In: Flaschka, A. & Barnard, A.J. (Eds.). *Chelates in analytical chemistry, vol. 2*. New York: Marcel Dekker.

Kumar, K.V., Sunand, K., Ashwini, K., Kumar, P.S., Vishnu, S. & Samala, A. (2017). Synthesis characterization and antibacterial studies of 4-aminoantipyrine schiff's bases. *Int. J. App. Pharm. Sci. & Res., 2*, 8 – 14.

Kwak, S.-Y., Lee, S., Choi, H.-R., Park, K.-C. & Lee, Y.-S . (2011). Dual effects of caffeoyl-amino acidyl-hydroxamic acid as an antioxidant and depigmenting agent. *Bioorg. & Med. Chem. Lett., 21* , 5155 – 5158.

Malladi, S., Isloor, A.M., Isloor, S., Akhila, D.S. & Fun, H.-K . (2013). Synthesis, characterization and antibacterial activity of some new pyrazole based Schiff bases. *Araboan J. Chem., 6*, 335 – 340.

Mohini, Y., Prasad, R.B.N., Karuna, M.S.L., Kumar, C.G., Poornima, M. & Sujitha, P. (2013). Synthesis of fatty acid Schiff base esters as potential antimicrobial and chemotherapeutic agents. *Med. Chem. Res., 22*, 4360 – 4366.

Nenadis, N. & Tsimidou, M. (2002). Observations on the estimation of scavenging activity pf phenolic compounds using rapid 1.1-diphenyl-2-picrylhydrazyl (DPP) tests. *J. Amer. Oil Chem. Soc., 79*, 1191 – 1195.

Olivares, C. & Solano, F. (2009). New insights into the active site structure and catalytic mechanism of tyrosinase and its related proteins. *Pigm. Cell Melanoma Res., 22*, 750–760.

Pannecouque, C., Daelemans, D. & De Clercq, E. (2008). Tetrazolium-based colorimetric assay for the detection of HIV replication inhibitors: revisited 20 years later. *Nat. Protoc., 3*, 427 – 434.

Petrus, M.L., Bein, T., Dingemans, T.J. & Docampo, P. (2015). A low cost azomethine-based hole transporting material for perovskite photovoltaics. *J. Mater. Chem. A., 3*, 12159 – 12162.

Popova, A., Christov, M., Raicheva, S. & Sokolova, E. (2004). Adsorption and inhibitive properties of benzimidazole derivatives in acid mild steel corrosion. *Corros. Sci., 46*, 1333 – 1350.

Raykova, R.N.,Carvalho, E., Manovski, L.S. , Danalev, D.L., Marinkova, D.A., Pajpanova, T.I., Yaneva, S.A. & Yotova, L.K. (2017). Kinetic of inhibition of lipoxygenase in presence of natural amino acid serine. *Bulg. Chem. Commun.,49, Special issue E*, 107 – 112.

Robb, D.A. (1984). Tyrosinase (pp. 207 – 240). In: Lontie, R. (Ed.). *Copper proteins and dopper enzymes: vol. II*. Boca Raton: CRC Press.

Santos, P.M.P., Antunes, A.M.M., Noronha, J., Fernandes, E. & Vieira, A.J.S.C. (2010). Scavenging activity of aminoantipyrines against hydroxyl radical. *Eur. J. Med. Chem., 45*, 2258 – 2564.

Sayed, G.H., Shiba, S.A., Radwan, A., Mohamed, S.M. & Kalil, M . (1992). Synthesis and reactions of some 6-aryl and 2, 6-diaryl-4-(4′-antipyrinyl)-2, 3, 4, 5-tetrahydropyridazin-3-ones and screening for their antibacterial activities. *Chinese J. Chem., 10*, 475 – 480.

Shi, L., Ge, H.-M., Tan, S.-H., Li, H.-Q., Song, Y.-C., Zhu, H.-L. & Tan, R.-X. (2007). Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. *Eur. J. Med. Chem., 42*, 558 – 564.

Shoaib, M., Rahman, G., Shah, S.W.A. & Umar, M.N. (2015). Synthesis of 4-aminoantipyrine derived Schiff bases and their evaluation for antibacterial, cytotoxic and free radical scavenging activity. *Bangladesh J. Pharmacol., 10*, 332 – 336.

Sondhi, S.M., Sharma, V.K., Singhal, N., Verma, R.P., Shukla, R., Raghubir, R. & Dubey, M.P. (2000). Synthesis and anti-inflammatory activity evaluation of some acridinyl amino antypyrine, acridinyl amino anthraquinone, acridino thiourea and thiazolino thiourea derivatives. *Phosphorus, Sulfur, Silicon & Relat. Elem., 156*, 21 – 33.

Sudo, K., Konno, K., Yokota, T. & Shigeta, S. (1994). A sensitive assay system screening antiviral compounds against herpes simplex virus type 1 and type 2. *J. Virol. Methods, 49* , 169 – 178.

Surh, Y.-J., Chun, K.-S., Cha, H.-H., Han, S.S., Keum, Y.-S., Park, K- K. & Lee, S.S. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-B activation. *Mutat. Res., 480 – 481*, 243 – 268.

Teng, Y., Liu, R., Li, C. & Zhang, H. (2011). Effect of 4-aminoantipyrine on oxidative stress induced by glutathione depletion in single human erythrocytes using a microfluidic device together with fluorescence imaging. *J. Hazard. Mater., 192*, 1766 – 1771.

Turan-Zitouni, G., Sivaci, M., Kiliç, F.S. & Erol, K. (2001). Synthesis of some triazolyl-antipyrine derivatives and investigation of analgesic activity. *Eur. J. Med. Chem., 36*, 685 – 689.

Vaghasiya, Y.K., Nair, R., Soni, M., Baluja S. & Chanda, S. (2004). Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine. *J. Serb. Chem. Soc., 69*, 991 – 998.

Windholz, M. & Budavari, S. (1983). The Merck index : an encyclopedia of chemicals, drugs, and biologicals. Rahway: Merck & Co.

Wong, A. (2002). A peappraisal of antipyretic and analgesic drugs . *WHO Pharm. Newsl. ,*No. 1, 15 – 16.

Yaneva, S.A. , Marinkova, D.A., Ilieva, L. ,Vezenkov, L.T., Yotova, L.K. & Danalev, D..L. (2017). Kinetic investigation and tyrosinase inhibition activity of peptide analogues of galanthamine. *Bulg. Chem. Commun., 49, Special issue E*, 64 – 67.

Yoshioka, S., Ogata, H., Shibazaki, T. & Ejima, A. (1981). The analgesic effects of the decomposition products of sulpyrine, N-[2-(5-hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine and Antipyrinyl-4-peroxide. *Chem. Pharm. Bull*, *29,* 1179 – 1181.

Yu, F., Jia, Y-L, Wang, H-F, Zheng, J., Cui, Y., Fang, X-Y., Zhang, L.-M. & Cheng, Q.-X. (2015). Synthesis of triazole Schiff's base derivatives and their inhibitory kinetics on tyrosinase activity. *PLoS ONE, 10*, art. no. 0138578.

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