

Palladium-catalyzed synthesis of novel tetra- and penta-cyclic biologically active benzopyran- and pyridopyran-containing heterocyclic systems

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Abstract

Syntheses of novel tetra- and penta-cyclic benzopyran and pyridopyran derivatives, *via* direct intramolecular arylation of 2-iodophenoxymethylarenes and 3-(2-bromo-pyridin-3-yloxymethyl)-benzo[4,5]imidazo[2,1-*b*]thiazole in the catalytic system Pd(OAc)₂ / Xantphos / Cs₂CO₃ / Ag₂CO₃ in toluene, and a one-pot bicatalytic method for 12*H*-[1]benzopyrano[3',4':4,5]thiazolo[3,2-*a*]benzimidazole directly from 3-chloromethylbenzo[4,5]imidazo[2,1-*b*]thiazole and 2-iodophenol, are described. This latter compound exhibits high cytotoxicity (MG-22A, 6 µg/mL) on the mouse hepatoma cancer cell line and low toxicity (LD₅₀, 1058 mg/kg) on the mouse Swiss albino embryo fibroblasts 3T3.

Keywords: Palladium catalysis, intramolecular arylation, phase transfer catalysis, fused benzothiazoles, imidazoles, benzopyrans, pyridopyrans, cytotoxicity

Introduction

Pyrans and their benzo derivatives are of interest as biologically active compounds.¹⁻³ The synthesis and reactions of pyrans and benzopyrans have been well reviewed.⁴⁻⁹ Recently the uses and properties of important natural and synthetic 2*H*-pyran-2-ones in organic synthesis were documented.^{8, 9} One of the earliest works describing the synthesis of the pyranothiazole ring from 3,5-dibromopyran-4-one and a thioamide was published in 1948.¹⁰ Some methods for the preparation of 2-substituted [1]benzopyrano[3,4-*d*]thiazol-4-ones and -imidazol-4-ones are documented.¹¹ More recently, the palladium-catalyzed synthesis of thiazolobenzopyran-2-ones from methyl 5-(2-allyloxyphenyl)thiazole-4-carboxylates was reported.¹² Benzopyranothiazole and benzopyranothiophene ring systems were prepared by thermal intramolecular 1,3-dipolar cycloaddition of 2-(prop-2-ynyloxy- and cyanomethyloxy)-3,5-diphenyl-4-hydroxythiazolium

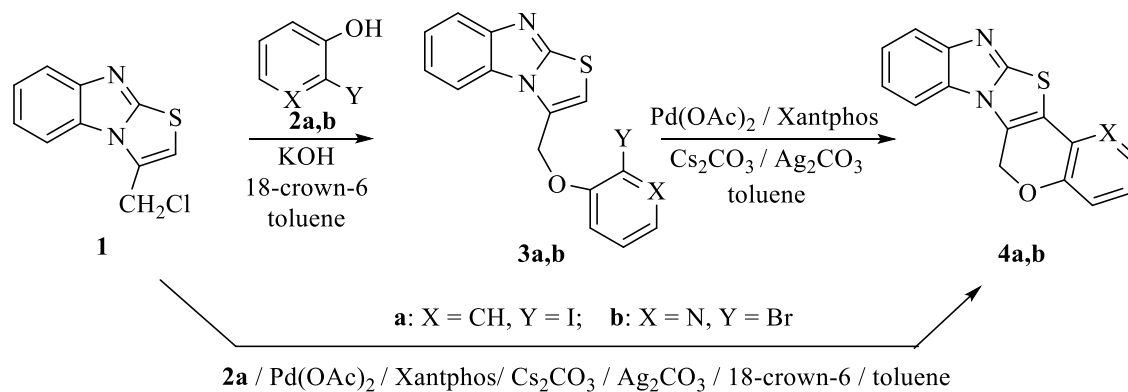
hydroxides.¹³ Benzopyranoimidazoles have been prepared by electrochemical reduction / rearrangement of benzopyranotriazines¹⁴ or by condensation of hydrazine with 4-oxochroman-3-carbaldehyde¹⁵.

An important modern development in the palladium-catalyzed synthesis of heterocyclic compounds using an intramolecular Heck-type reaction was recently highlighted in some reviews.¹⁶⁻¹⁹ Regioselective functionalization of the imidazole ring by transition metal-catalyzed C-N and C-C bond formation was reported in details by Rossi *et al.*²⁰ Interestingly, that the pyrano[3',4':4,5]imidazo[1,2-*a*]pyridin-1-one ring system has been constructed by Cu(I)-catalyzed cross-coupling and heterocyclization reactions of halogenated imidazopyridine-carboxylic acids in the presence of terminal alkynes.²¹

Our interest in polycyclic compounds containing imidazothiazole and related fragments was prompted by the wide range of biological activity of these heterocyclic systems.²²⁻²⁴ Furthermore, the synthesis of tetra- and penta-cyclic imidazolo- and thiazolo-benzopyran derivatives has not been investigated until now and so is the main aim of the present work. Beside this, selected stable compounds (**4a**, **10** and **13**) were tested as cytotoxic agents.

Results and Discussion

Synthesis of the novel polycyclic compounds **4a,b**, **7**, **10** and **13** was carried out in two steps. Alkylation of 2-iodophenol (**2a**) or 2-bromo-3-hydroxypyridine (**2b**) with chloromethylhetarenes **1**, **5**, **8**, **11**²⁴ was successfully achieved under phase transfer catalytic conditions – solid KOH / 18-crown-6 / toluene. Intermediates **3a,b**, **6**, **9** and **12** were isolated in 21-87 % yields. (Schemes 1-4)



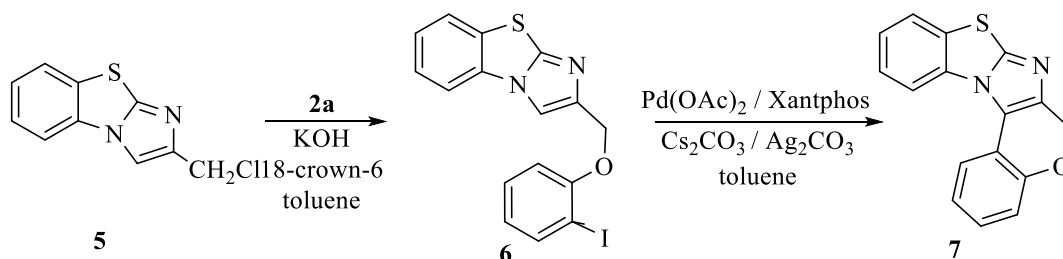
Scheme 1

The influence of catalyst, ligand and additive on the intramolecular Heck-type cyclization was studied in detail. Initially, we examined direct intramolecular arylation of 2-iodophenoxy-methylhetarene **3a** using 10 mol.% Pd(PPh₃)₄ as catalyst and 2 eq. Cs₂CO₃ as base. No product

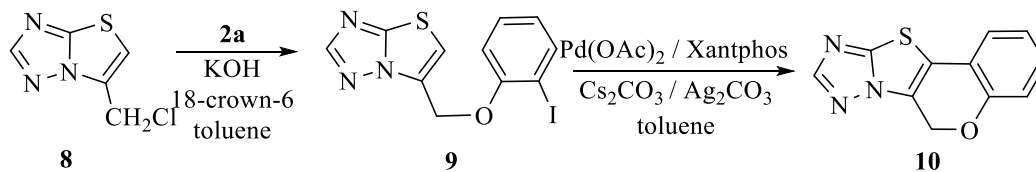
was found under these conditions. The use of the system 10 mol.% Pd(OAc)₂ / 20 mol.% Xantphos / Cs₂CO₃ (2 eq.) / toluene provided the polycycle **4a** in a low (17%) yield with 55% conversion. The high activity of Ag₂CO₃ as an additive in Rh-catalyzed arylation of hetarenes was recently demonstrated.²⁵ The addition of 0.5 eq. Ag₂CO₃ to the system Pd(OAc)₂ (10 mol.%) / Xantphos (20 mol.%) / Cs₂CO₃ (2 eq.) / toluene furnished the desired product **4a** in improved (69 %) yield with full conversion of the starting material **3a**. All catalytic systems in the presence of Ag₂CO₃ were more active.

The polycycle **4a** was also obtained directly from 3-chloromethylbenzo[4,5]imidazo[2,1-*b*]thiazole (**1**) and 2-iodophenol (**2a**) in a *one-pot* synthesis using the bicatalytic system Pd(OAc)₂ (10 mol.%) / Xantphos (20 mol.%) / Cs₂CO₃ (3 eq.) / Ag₂CO₃ (0,5 eq.) / 18-crown-6 (10 mol.%), but in lower yield (25%) than the two step synthesis provided.

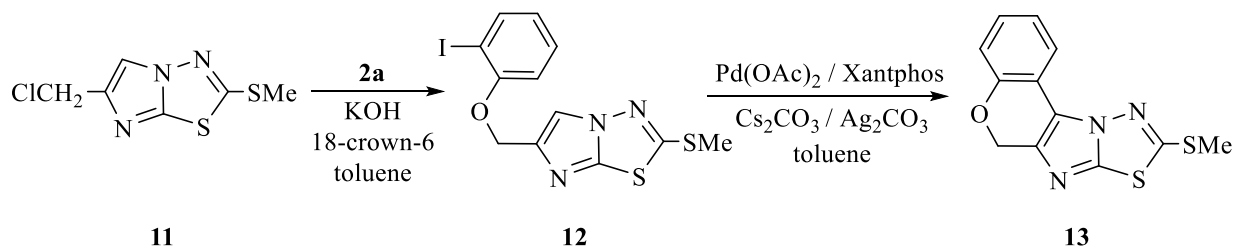
The catalytic system Pd(OAc)₂ (10 mol.%) / Xantphos (20 mol.%) / Cs₂CO₃ (2 eq.) / Ag₂CO₃ (0,5 eq.) / toluene, as the most active, was used for the preparation of the polycyclic compounds **4b** (Scheme 1), **7** (Scheme 2), **10** (Scheme 3) and **13** (Scheme 4). The products **4a,b**, **7**, **10** and **13** were isolated by column chromatography in 31-63% yields.



Scheme 2



Scheme 3



Scheme 4

The structure of compound **13** was confirmed by X-ray structural data. Needle like crystals of compound **13** suitable for intensity measurement were grown from chloroform. The entire molecule of polycycle **13** is essentially planar (Figure 1). The maximal deviation from the least squares mean plane drawn through all non-hydrogen atoms of the molecule is 0.398(2) Å for the O5 atom. The molecule contains four condensed rings, the 1,3,4-thiadiazole (A), imidazole (B), pyran (C) and benzene (D). Rings A, B and D are planar within 0.002 Å, 0.002 Å and 0.01 Å respectively. The pyran ring (C) adopts a twist-half-chair conformation where atoms C6, C6a, C10b and C10c form a strict plane (± 0.005 Å), while the O5 and C4a atoms deviate from this plane by 0.564(4) Å and 0.258(4) Å respectively. The thiadiazolo-imidazole system (A+B) forms a dihedral angle of 9.5(1)° with the benzene ring (D).

Bond lengths in compound **13** are in good agreement with the crystal structure of methyl 2-chloro-8-oxo-6*H*,8*H*-[1]benzopyrano[4',3':4,5]imidazo[2,1-*b*][1,3]thiazine-10-carboxylate²⁶ having three similar condensed rings (B+C+D fragment), and of 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-2-sulfonamide²⁷ with a similar thiadiazolo-imidazole system (A+B). Intermolecular contacts are all of the van der Waals type.

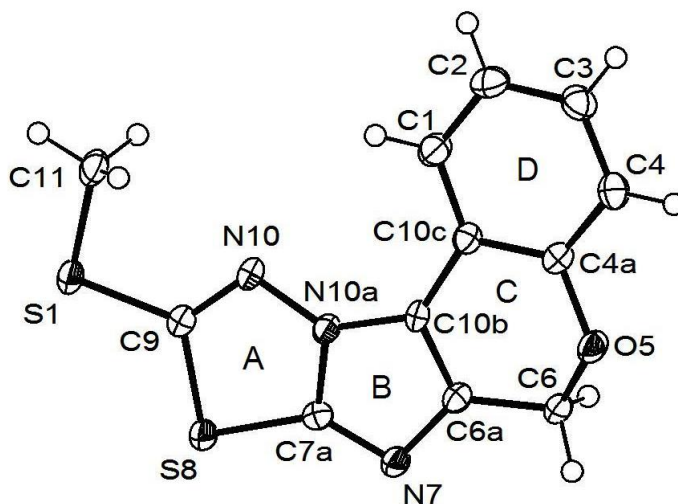


Figure 1. View of the molecule **13** with atomic numbering and ring labels.

Table 1. Crystal data of the compound **13**

Empirical formula	C ₁₂ H ₉ N ₃ OS ₂
Formula weight	275.34
Temperature (K)	180(2)
Crystal system	orthorombic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>a</i> (Å)	3.9926(1)
<i>b</i> (Å)	10.2615(3)
<i>c</i> (Å)	27.847(1)
Volume (Å ³)	1140.88(6)
<i>Z</i>	4
Calc. density (Mg/m ⁻³)	1.603
Crystal size (mm)=	0.4 × 0.1 × 0.1
Crystal colour	Colorless
θ range (°)	2.12 - 27.10
Index ranges	<i>h</i> -5 → 5 <i>k</i> -12 → 13 <i>l</i> -34 → 35
Data / Restraints / Parameters	2279 / 0 / 163
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	0.0326 / 0.0670
<i>R</i> indices (all data)	0.0417 / 0.0707
Hydrogen atoms treatment	Constrained
Largest diff. peak and hole (e.Å ⁻³)	0.274 and -0.248

Table 2. Cytotoxicity of polycyclic compounds **4a**, **10** and **13** IC₅₀ (μg/mL)

Compound	HT-1080, IC ₅₀	MG-22A, IC ₅₀	3T3, LD ₅₀ , mg/kg
4a	40	6	1058
10	20	28	1100
13	50	78	1487

Cytotoxic activity of compounds **19** and **23** was tested *in vitro* on two monolayer tumor cell lines: MG-22A and HT-1080 (Table 2). Compound **4a** exhibit high activity on the mouse hepatoma (MG-22A, 6 μg/mL) cancer cell line. However, on the human fibrosarcoma cell line this compound is essentially inactive. Polycyclic compound **10** exhibit middle activity on both cancer cell lines. Compound **13** is inactive on the MG-22A and HT-1080 cancer cell lines. Interestingly, that toxicity of compounds **4a**, **10** and **13** (LD₅₀, 1058-1487 mg/kg) detected on the mouse normal fibroblasts is not high.

Conclusions

In summary, we have developed a facile method for synthesis of novel imidazole and thiazole containing benzopyran and pyridopyran derivatives *via* intramolecular cyclization of corresponding 2-iodophenoxy(or bromopyridin-3-yloxy)methylhetarenes in the catalytic system Pd(OAc)₂ / Xantphos / Cs₂CO₃ / Ag₂CO₃ / toluene. 12*H*-[1]Benzopyrano[3',4':4,5]thiazolo[3,2-*a*]benzimidazole (**4a**) exhibit high cytotoxicity on the mouse hepatoma (MG-22A, 6 µg/mL) cancer cell line and low toxicity on mouse Swiss Albino embryo fibroblasts (3T3, LD₅₀ 1058 mg/kg).

Experimental Section

General. ¹H and ¹³C NMR spectra were recorded on a Varian Mercury BB 400 MHz in CDCl₃ using HMDSO as internal standard. LC-MS spectra were recorded on Alliance Waters 2695 instrument and Waters 3100 mass detector. Column chromatography was performed with silica gel 0,035-0,070 nm (Acros). X-Ray diffraction data was collected using Nonius KappaCCD single crystal diffractometer (Bruker AXS) (MoKα₁ - radiation, graphite monochromator). The structure was solved by SIR2004²⁸ and refined by SHELXL97²⁹ programs. Rms deviation of fitted atoms = 0.0066. 3-Chloromethylbenzo[4,5]imidazo[2,1-*b*]thiazole (**1**), 2-chloromethylbenzo[d]imidazo[2,1-*b*]thiazole (**5**), 6-chloromethylthiazolo[3,2-*b*][1,2,4]triazole (**8**), 6-chloromethyl-2-methylsulfanylimidazo[2,1-*b*][1,3,4]thiadiazole (**11**) were obtained by the procedure described in article.²⁴ All prepared compounds are new and were characterized by melting point, LC-MS, HRMS, ¹H NMR and ¹³C NMR spectra.

General procedure for synthesis of 2-iodophenoxymethylhetarenes **3a**, **6**, **9**, **12** and 3-(2-bromopyridin-3-yloxymethyl)-benzo[4,5]imidazo[2,1-*b*]thiazole (**3b**)

Solid pulverized KOH (0.98 g, 4.5 mmol) was added to solution of chloromethyl derivatives **1**, **5**, **8** or **11** (4 mmol), 2-iodophenol (**2a**) (0.88 g, 4 mmol) or 2-bromo-3-hydroxypyridine (**2b**) (0.87 g, 4 mmol), 18-crown-6 (0.1g, 0.4 mmol) in toluene (20 mL). Reaction mixture was refluxed for 2 h, cooled to room temperature, filtered and solvent was removed under reduced pressure. The products were purified using flash chromatography (silica, ethyl acetate). Spectroscopic characteristics.

3-(2-Iodophenoxymethyl)-benzo[4,5]imidazo[2,1-*b*]thiazole (3a**).** 66% yield; mp 165-166 °C; LC-MS, 407 (M⁺+1); ¹H NMR δ (□ppm□): 5.40 (s, 2H, CH₂), 6.82 (t, 1H, *J* = 7.6 Hz, 4'-H), 6.86 (s, 1H, 2-H), 6.96 (d, 1H, *J* = 8.4 Hz, 6'-H), 7.26 (t, 1H, *J* = 8.0 Hz, 5'-H), 7.32-7.41 (m, 2H, 6-H and 7-H), 7.80-7.84 (m, 3H, 5-H, 8-H and 3'-H); ¹³C NMR δ (□ppm□): 63.80 (CH₂), 86.97, 109.97, 111.95, 113.15, 119.17, 121.19, 123.61, 124.15, 128.48, 129.55, 129.78, 140.09, 148.40, 156.20, 156.81.

3-(2-Bromopyridin-3-yloxymethyl)-benzo[4,5]imidazo[2,1-*b*]thiazole (3b). 44% yield; mp 204-205 °C; LC-MS, 361 (M^{+1}); $^1\text{H NMR } \delta$ ($\square\text{ppm}\square$): 5.75 (s, 2H, CH_2), 7.26 and 7.35 (both t, 2H, $J = 7$ Hz, 6-H and 7-H), 7.50 (m, 1H, 5'-H), 7.52 (s, 1H, 2-H), 7.70 (d, 1H, $J = 8$ Hz, 4'-H), 7.85-7.90 (m, 2H, 5-H and 8-H), 8.05 (d, 1H, $J = 5$ Hz, 6-H'); $^{13}\text{C NMR } \delta$ ($\square\text{ppm}\square$): 62.57 (CH_2), 112.50, 112.60, 118.38, 120.73, 121.86, 123.27, 124.28, 127.94, 129.41, 131.72, 142.22, 147.77, 150.59, 156.29.

2-(2-Iodophenoxymethyl)-benzo[*d*]imidazo[2,1-*b*]thiazole (6). 21% yield; mp 175-177 °C; LC-MS, 407 (M^{+1}); $^1\text{H NMR } \delta$ ($\square\text{ppm}\square$): 5.27 (s, 2H, CH_2), 6.74 (t, 1H, $J = 7.2$ Hz, 4'-H), 7.03 (d, 1H, $J = 8.4$ Hz, 6'-H), 7.26-7.46 (m, 3H, 5'-H, 6-H and 7-H), 7.60, 7.69 and 7.79 (all d, 3H, $J = 8.0$ Hz, 3'-H, 5-H, 8-H), 7.85 (s, 1H, 3-H); $^{13}\text{C NMR } \delta$ ($\square\text{ppm}\square$): 66.37 (CH_2), 86.73, 110.07, 112.80, 112.97, 115.19, 122.94, 124.33, 124.91, 126.14, 129.53, 138.40, 139.45, 143.99, 147.47, 157.11.

6-(2-Iodophenoxymethyl)-thiazolo[3,2-*b*][1,2,4]triazole (9). 87% yield; mp 121-122 °C; LC-MS, 358 (M^{+1}); $^1\text{H NMR } \delta$ ($\square\text{ppm}\square$): 5.38 (s, 2H, CH_2), 6.80 (t, 1H, $J = 8.0$ Hz, 4'-H), 6.96 (d, 1H, $J = 8.4$ Hz, 6'-H), 7.24 (d, 1H, $J = 1.6$ Hz, 5-H), 7.34 (t, 1H, $J = 8.0$ Hz, 5'-H), 7.82 (d, 1H, $J = 8.0$ Hz, 3'-H), 8.19 (d, 1H, $J = 1.2$ Hz, 2-H); $^{13}\text{C NMR } \delta$ ($\square\text{ppm}\square$): 63.39 (CH_2), 86.67, 111.40, 112.87, 123.97, 128.48, 129.72, 139.76, 156.30, 156.35, 156.84.

6-(2-Iodophenoxymethyl)-2-methylsulfanylimidazo[2,1-*b*][1,3,4]thiadiazole (12). 42% yield; mp 124-125 °C; LC-MS, 404 (M^{+1}); $^1\text{H NMR } \delta$ ($\square\text{ppm}\square$): 2.73 (s, 3H, CH_3), 5.19 (s, 2H, CH_2), 6.72 (t, 1H, $J = 8.8$ Hz, 5'-H), 6.99 (d, 1H, $J = 8.4$ Hz, 6'-H), 7.29 (t, 1H, $J = 8.4$ Hz, 4'-H), 7.78 (d, 1H, $J = 7.6$ Hz, 3'-H), 7.83 (s, 1H, 5-H); $^{13}\text{C NMR } \delta$ ($\square\text{ppm}\square$): 16.07 (Me), 66.80 (CH_2), 86.80, 112.54, 112.95, 122.82, 129.44, 139.46, 142.00, 144.21, 157.06, 161.44.

General procedure for synthesis of polycyclic compounds 4a,b, 7, 10 and 13

Mixture of 2-iodophenoxymethylhetarenes **3a**, **6**, **9**, **12** or 3-(2-bromo-pyridin-3-yloxymethyl)-benzo[4,5]imidazo[2,1-*b*]thiazole **3b** (0.49 mmol), $\text{Pd}(\text{OAc})_2$ (0.011 g, 0.049 mmol), Xantphos (0.057 g, 0.098 mmol), anhydrous Cs_2CO_3 (0.32 g, 0.98 mmol) and Ag_2CO_3 (0.068 g, 0.25 mmol) in dry toluene (10 mL) was heated at 120 °C for 24 h in glass reactor under argon. Reaction mixture was filtered and solvent was removed under reduced pressure. The products were purified using flash chromatography (silica, ethyl acetate : hexane (1:1)). Spectroscopic characteristics:

12*H*-[1]Benzopyrano[3',4':4,5]thiazolo[3,2-*a*]benzimidazole (4a). 69% yield; mp >230 °C; LC-MS, 279 (M^{+1}); $^1\text{H NMR } \delta$ ($\square\text{ppm}\square$): 5.80 (s, 2H, CH_2), 6.96 (d, 1H, $J = 8.0$ Hz, 4-H), 7.02 (t, 1H, $J = 8.0$ Hz, 2-H), 7.10 (d, 1H, $J = 7.6$ Hz, 1-H), 7.18 (t, 1H, $J = 7.6$ Hz, 3-H), 7.26 and 7.36 (both t, 2H, $J = 8.0$ Hz, 8-H and 9-H), 7.53 and 7.79 (both d, 2H, $J = 8.0$ Hz, 7-H and 10-H); $^{13}\text{C NMR } \delta$ ($\square\text{ppm}\square$): 63.24 (CH_2), 110.14, 116.26, 116.28, 116.38, 118.13, 119.72, 121.57, 121.79, 122.61, 122.81, 122.83, 123.75, 123.77, 129.25, 129.38; HRMS: m/z [$M+H$] $^+$ calcd for $\text{C}_{16}\text{H}_{11}\text{N}_2\text{OS}$: 279.0592; found 279.0603.

6*H*-Pyrido[3'',2'':2',3']pyrano[4',5':5,4]thiazolo[3,2-*a*]benzimidazole (4b). 49% yield; mp >230 °C; LC-MS, 280 (M^{+1}); $^1\text{H NMR } \delta$ ($\square\text{ppm}\square$): 5.85 (s, 2H, 6-H), 7.03 (m, 1H, 3-H), 7.15

(d, 1H, $J = 8.4$ Hz, 4-H), 7.24 and 7.34 (both t, 2H, $J = 8.0$ Hz, 8-H and 9-H), 7.47 and 7.76 (both d, 2H, $J = 8.0$ Hz, 7-H and 10H), 9.09 (d, 1H, $J = 4.8$ Hz, 2-H); ^{13}C NMR δ ($\square\text{ppm}\square$): 63.87 (CH_2), 110.08, 117.63, 119.75, 121.69, 122.26, 123.47, 124.04, 124.84, 129.17, 119.97, 137.87, 143.00, 147.99, 156.76; HRMS: m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{10}\text{N}_2\text{OS}$: 280.0545; found 280.0531.

6H-[1]Benzopyrano[3',4':4,5]imidazo[2,1-*b*]benzothiazole (7). 63% yield; mp 153-155 °C; LC-MS, 279 (M^++1); ^1H NMR δ ($\square\text{ppm}\square$): 5.29 (s, 2H, 6-H), 7.07-7.21 and 7.26-7.49 (m, 5H, 2-H, 3-H, 4-H, 10-H and 11-H), 7.72 (m, 2H, 1-H, 12-H), 8.14 (t, 1H, $J = 8.4$ Hz, 9-H); ^{13}C NMR δ ($\square\text{ppm}\square$): 60.36 (CH_2), 114.16, 114.80, 117.98, 118.09, 121.80, 122.29, 124.49, 124.79, 125.93, 127.61, 130.00, 132.79, 142.08, 149.43, 152.51; HRMS: m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{11}\text{N}_2\text{OS}$: 279.0592; found 279.0584.

6H-[1]Benzopyrano[3',4':4,5]thiazolo[3,2-*b*][1,2,4]triazole (10). 31% yield; mp 131-132 °C; LC-MS, 230 (M^++1); ^1H NMR δ ($\square\text{ppm}\square$): \square 5.58 (s, 2H, 6-H), 6.97 (d, 1H, $J = 8.4$ Hz, 4-H), 7.01 (t, 1H, $J = 7.6$ Hz, 2-H), 7.16 (d, 1H, $J = 8.0$ Hz, 1-H), 7.22 (t, 1H, $J = 8.0$ Hz, 3-H), 8.13 (s, 1H, 8-H); ^{13}C NMR δ ($\square\text{ppm}\square$): 62.73 (CH_2), 116.75, 117.54, 120.59, 121.53, 122.58, 122.81, 130.07, 151.90, 155.87, 156.18; HRMS: m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{11}\text{H}_8\text{N}_3\text{OS}$: 230.0388; found 230.0399.

9-Methylsulfanyl-6H-[1]Benzopyrano[3',4':4,5]imidazo[2,1-*b*][1,3,4]thiadiazole (13). 47% yield; mp 153-155 °C; LC-MS, 276 (M^++1); ^1H NMR δ ($\square\text{ppm}\square$): 2.82 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 6.93 (d, 1H, $J = 8.0$ Hz, 4-H), 7.00 and 7.73 (both t, 2H, $J = 7.6$ Hz, 2-H and 3-H), 7.73 (d, 1H, $J = 7.6$ Hz, 1-H); ^{13}C NMR δ ($\square\text{ppm}\square$): 16.24 (CH_3), 67.72 (CH_2), 115.70, 116.50, 120.73, 121.64, 121.65, 128.11, 136.09, 145.30, 151.56, 161.82; HRMS: m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{10}\text{N}_3\text{OS}_2$: 276.0265; found 276.0256.

\square **In vitro cytotoxicity assay.** Monolayer tumor cell lines –HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), 3T3 (mouse Swiss Albino embryo fibroblasts), - were cultured in standard medium (Dulbecco's modified Eagle's medium; DMEM) and supplemented with 10% fetal bovine serum ("Sigma"). Tumor cell lines were obtained from the ATCC. About 10×10^4 cells ml^{-1} were placed in 96-well plates immediately after compounds were added to the wells; the volume of each plate was 200 μl . The control cells without test compounds were cultured on separate plate. The plates were incubated for 72h, 37 °C, 5% CO_2 . The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)^{30, 31}. LD_{50} was tested according "Alternative Toxicological Methods".³² The program Graph Pad Prism[®] 3.0 was used for calculations ($r^2 < 0.05$).

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