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Synthesis of novel Anticancer Ruthenium-Arene Pyridinylmethylene scaffolds *via* Three-Component reaction

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Abstract: A novel three components approach for the synthesis of bioactive Ru–arene pyridinylmethylene complexes has been developed using pyridine carboxaldehyde, amino pyridine and dichloro (*p*-cymene) ruthenium (II) dimer as starting materials. These scaffolds were screened for their anticancer activity against breast cancer (MCF7) & human Epitheloid Cervix Carcinoma (HeLa) cell line. It was established that compounds [(η6-*p*cymene)RuCl(κ2-*N*,*N*-(3,5-dinitro-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]PF_6 (**4o**), [(η6-*p*cymene)RuCl(κ2-*N*,*N*-N-(3,5-Dibromo-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]PF_6 (**4c**), [(η6-*p*cymene)RuCl(κ2-*N*,*N*-(3,5-dibromo-6-methylpyridin-2-yl)-pyridin-2-ylmethylene-amine)]PF_6 (**4j**) & [(η6-*p*cymene)RuCl(κ2-*N*,*N*-3(3-Bromo-5-methyl-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]PF_6 (**4b**) were significantly active against both the cell lines.

Keywords: *Ruthenium dimer, Ruthenium-Arene Pyridinylmethylene scaffolds, three component reactions, anticancer activity, fluorescence profile, toxicity study*

Usually, the research in drug discovery has been focused on the development of organic molecules as pharmacophores. However, their limited structural diversity makes it difficult for them to access other scaffolds to extent the whole biologically relevant chemical space,[1–3] Therefore, investigation of the structurally unique potentially valuable unexplored chemical spaces is highly warranted. Nowadays, transition-metal scaffolds have been developed as potential drug candidates. They can eagerly accommodate higher coordination number and consequently access different molecular geometries which are not possible with pure organic scaffolds[4].

The first successful transition metal scaffold, cisplatin, was discovered and approved by Food Drug and Administration (FDA) for the treatment of ovarian and testicular cancer.[5]

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Nevertheless, the attempt of fighting drug resistance and rigorous side effects throughout the treatment are the major drawbacks for cisplatin & its congeners.[6] Hence the development of complex containing metals other than platinum have been focused in our current research. During the last three decades, ruthenium (II) arene complexes occupy a prominent position among the various metal complexes explored for anticancer activity.[7] Moreover, two ruthenium compounds are under advanced clinical evaluation as anticancer drugs named as NAMI-A[8] and KP1019[9]. A number of interesting ruthenium (II) complexes bearing a π-bonded arene ligand has already been developed which show promising anticancer activity,[10-11] even in cells that had become resistant to cisplatin, for instance Sadler's compounds containing N,N chelating ligands.[12,13] Besides this, some of the Dyson's RAPTA compounds containing pta ligand have shown antimetastatic activity.[14,15] It was also established that some ruthenium compounds bind to DNA more strongly and are less affected by cell repair mechanisms compared to cisplatin.[16-18] There are several properties that makes ruthenium complexes are well accepted for medicinal applications such as their rate of ligand exchange, the range of accessible oxidation states, aqueous solubility and stability in biological environment, and the ability of ruthenium to mimic iron in binding to certain biological molecules.[19] In addition, a η^6–arene moiety stabilizes the oxidation state of metal ion and may assist it's transportation through cell membrane.[20] The three remaining coordination sites can be occupied with other ligands, forming a "piano stool" geometry, typical for the organoruthenium (II) complexes.[21,22] The leaving group in metal complex endures easy dissociation which allow the metal ion to coordinate with the target molecule. The reactivity of the complex with different biomolecules was preserved by ancillary bidentate ligand (κ^2-N, N-L) through hydrogen bonding interaction. Currently, the metals are coordinated with bioactive organic ligands to improve a biological activity which seems to be a

promising strategy in drug discovery.[23] In the recent literature, it was established that ruthenium complexes of organic drugs can overcome the resistance developed by the microbe to the organic compound alone.[24] Besides this, a combination of ruthenium-arene moiety with different chloroquinoline derivatives led to complexes which exhibited two to five fold more active than chloroquine against drug resistant Plasmodium parasites.[25] Recently, we have discovered a series of ruthenium-quinolinol complexes which played as potent antimicrobial agent against various gram positive & gram negative bacteria.[24] In addition, Mun Juinn Chow et. al. has reported a series of ruthenium-arene shiff base complexes which showed the low IC_{50} values in inhibition of cell viability against a breast cancer cell line MCF7, a human ovarian cell line A2780 and A2780cisR[26]. Despite of encouraging preliminary results, still there are questions about effectiveness of ruthenium complexes. These studies pave the way for the development of ruthenium-arene complexes as the next generation of metal-based anticancer drugs.

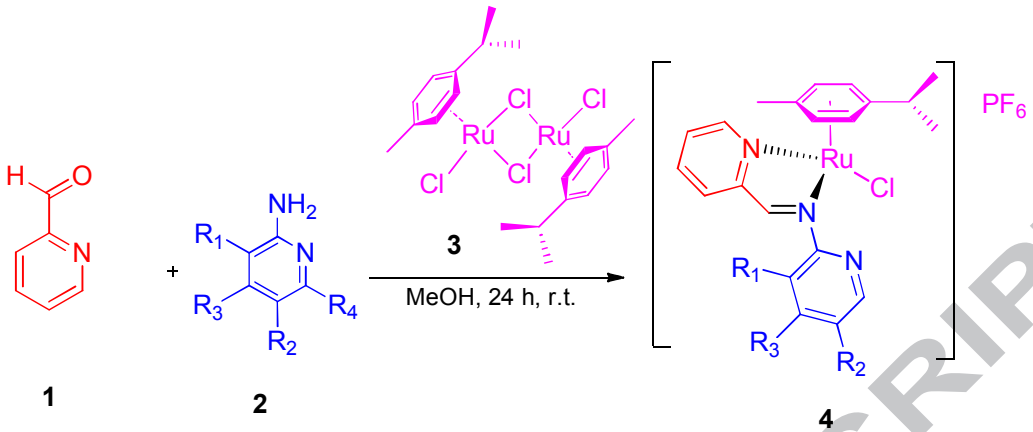
The multicomponent reactions and combinatorial approaches are impending tools for designing of diversified compound libraries[27, 28] for drug screening although they have not been usually applied to metal-based drug discovery. As a consequence, we have spotlighted in the synthesis of some novel organoruthenium scaffolds *via* multicomponent approach. Herein, we have discovered a simple and efficient approach for the synthesis of novel Ruthenium–arene pyridinylmethylene scaffolds *via* three component assembly and evaluated *in vitro* anticancer activity.

Our intention was to synthesize a library of novel Ru–arene complexes with ease of purification and operational simplicity *via* a one pot three-component approach. Initially, we have treated Pyridine-2-carboxaldehyde (**1**) with 2-aminopyridine (**2a**) in Methanol. But the formation of imine is irreversible as it hydrolyzed in aqueous medium. Thus, we prompted in multi-component approach for the synthesis of Ruthenium-arene pyridinylmethylene complexes. The reaction involves three components, exclusively, Ru–arene dimer (**3**), Pyridine-2-carboxaldehyde (**1**), and 2-aminopyridine (**2a**). Imine condensation between aldehyde and

aminopyridine forms a Schiff-base ligand that is predisposed for metal chelation. Such ligands are usually weakly stable in aqueous environment without metal chelation because of hydrolysis of the imine bond.

At the outset, dichloro *p*-cymene Ruthenium (II) dimer (**3**) was dissolved in methanol, and subsequently, 2 equivalents of Pyridine-2-carboxaldehyde (**1**) and 2-aminopyridine (**2a**) was added to the reaction mixture and stirred for 24 hours in ambient temperature. The progress of the reaction was monitored by TLC. A complete color change was observed after 24 hours of reaction. Consequently, the solvent methanol was evaporated and crude product was recrystallized from 5% methanol in diethyl ether *via* vapor diffusion method.

The structure of this complex (**4a**) was fully characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS. Commonly, in ^{1}H NMR the arene protons in Ru(II)–arene complexes were shifted downfield relative to the corresponding starting Ru(II) dimers. There were also characteristic changes in the splitting pattern of the protons in the arene ligand. Upon ligand coordination, the C_{2v} symmetry of the complex was interrupted. In $CDCl_3$, **4a** exhibited two separate doublets (σ 1.08 & 1.28 ppm) for cymene CH in the ^{1}H NMR spectra due to the formation of enantiomers. Similarly, the arene CH protons displayed four separate doublets (σ 5.63-6.07 ppm). The characteristic peak of imine was exhibited as singlet (σ 10.13 ppm) in ^{1}H NMR spectra.[29] In ^{13}C NMR spectra, the characteristic peaks of three methyl carbon were exhibited in the range of σ 21-28 ppm. Likewise, the characteristic ruthenium isotopic pattern in mass spectra clearly indicates the quantitative formation of ruthenium complex. Once the reaction condition for the synthesis of **4a** was optimized, the scope of this chemical approach was extended for the synthesis of various Ru-arene complexes (**4a-r**). The structures were confirmed by their ^{1}H NMR, ^{13}C NMR & ESI-MS. The scheme of this general reaction was depicted below. (**Scheme 1**)



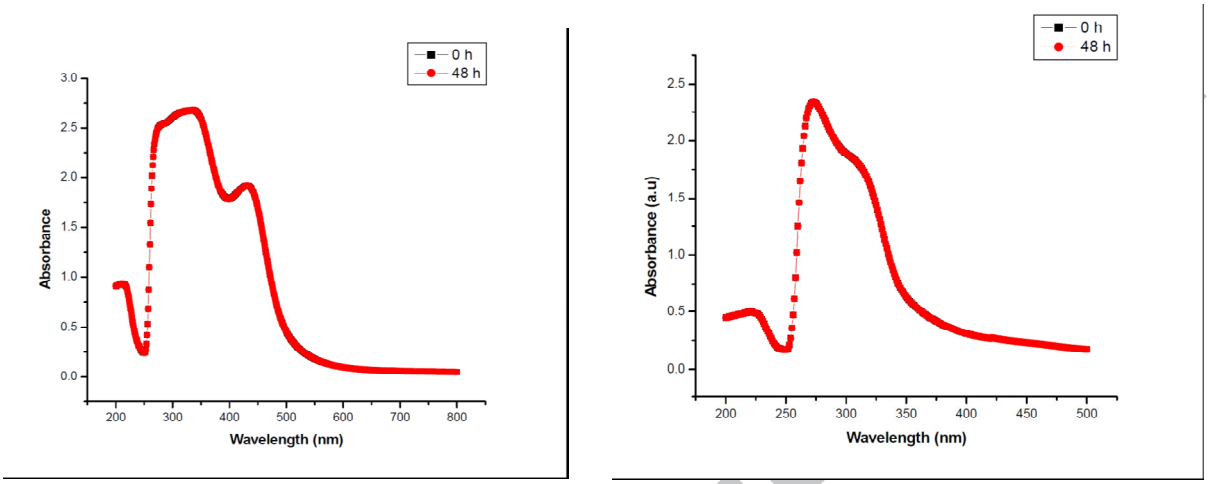
a. R_1 = H, R_2 = H, R_3 = H, R_4 = H
b. R_1 = Br, R_2 = Me, R_3 = H, R_4 = H
c. R_1 = Br, R_2 = Br, R_3 = H, R_4 = H
d. R_1 = H, R_2 = Br, R_3 = H, R_4 = H
e. R_1 = Me, R_2 = H, R_3 = H, R_4 = H
f. R_1 = H, R_2 = H, R_3 = Me, R_4 = H
g. R_1 = OH, R_2 = H, R_3 = H, R_4 = H
h. R_1 = H, R_2 = Me, R_3 = H, R_4 = H
i. R_1 = H, R_2 = Cl, R_3 = H, R_4 = H
j. R_1 = Br, R_2 = Br, R_3 = H, R_4 = Me
k. R_1 = Cl, R_2 = Cl, R_3 = H, R_4 = H
l. R_1 = H, R_2 = I, R_3 = H, R_4 = H
m. R_1 = NO_2, R_2 = Br, R_3 = Me, R_4 = H
n. R_1 = H, R_2 = Br, R_3 = Me, R_4 = H
o. R_1 = NO_2, R_2 = NO_2, R_3 = H, R_4 = H
p. R_1 = H, R_2 = NO_2, R_3 = H, R_4 = H
q. R_1 = NO_2, R_2 = Br, R_3 = H, R_4 = H
r. R_1 = H, R_2 = H, R_3 = H, R_4 = Me

Scheme-1 Possible route for the synthesis of complex **4**

The Ru–arene pyridinylmethylene complex (**4a-r**) assumes the pseudo-octahedral geometry as usual of organoruthenium species, wherein the *p*-cymene ring is π-bonded to the ruthenium ion. The remaining three coordination sites of ruthenium are occupied by the chlorido ligand and pyridinylmethylene ligand.

While DMSO has been used as a co-solvent in biological system, we investigated the stability of the Ru–arene pyridinylmethylene complex (**4b**) in DMSO-*d6* solution using ^{1}H NMR (see ESI†). Screening these solutions along the time, no changes were observed in the NMR signals. The stability of the complex **4b** in DMSO in presence of phosphate buffers with pH 8.0 was monitored by UV-Vis spectroscopy over a period of 24 h. The UV-Vis peak profile remains unchanged with the λ_{max} value, thus suggesting that compound is stable in DMSO (**Figure 1a**). Likewise, in the presence of 0.1 mM GSH, complex **4b** was not displaying any shift in UV-Vis

spectroscopy, which recommends that compound **4b** might be stable in intercellular thiol (**Figure 1b**).



(a) **(b)**

Figure 1. UV-Vis spectrum of (a) **4b** (c = 2×10^{-4} M) in 5% DMSO in phosphate buffer pH = 8 (b) **4b** (c = 2×10^{-4} mol L^{-1}) in 0.1 mM GSH in DMSO.

The fluorescence spectra of **4a-r** were measured at concentrations of $5x10^{-7}$molL^{-1} in different solvents. It was observed that compounds are not displaying enough fluorescence in methanol (**Figure S1**, see ESI†). However, compounds have been displayed excellent fluorescence in DMSO and dichloromethane (**Figure 2**, see ESI†). The fluorescence excitation wavelengths (λex/nm) and maximum emission wavelength is reported in **Table 1**. It was observed that compounds **4d**, **4g** and **4h** exhibited comparable fluorescence in both the solvents. Whereas, compounds **4a**, **4c** and **4i-r** exhibited 1.5-2 folds more fluorescence in DMSO than CH_2Cl_2. Interestingly, compounds **4b** and **4e** were not showing any remarkable fluorescence in DMSO. Nevertheless, compound **4e** is exhibited 1000 folds more fluorescence in CH_2Cl_2. Likewise, compounds **4b** and **4f** are exhibited 10 times more fluorescence in CH_2Cl_2 than DMSO.

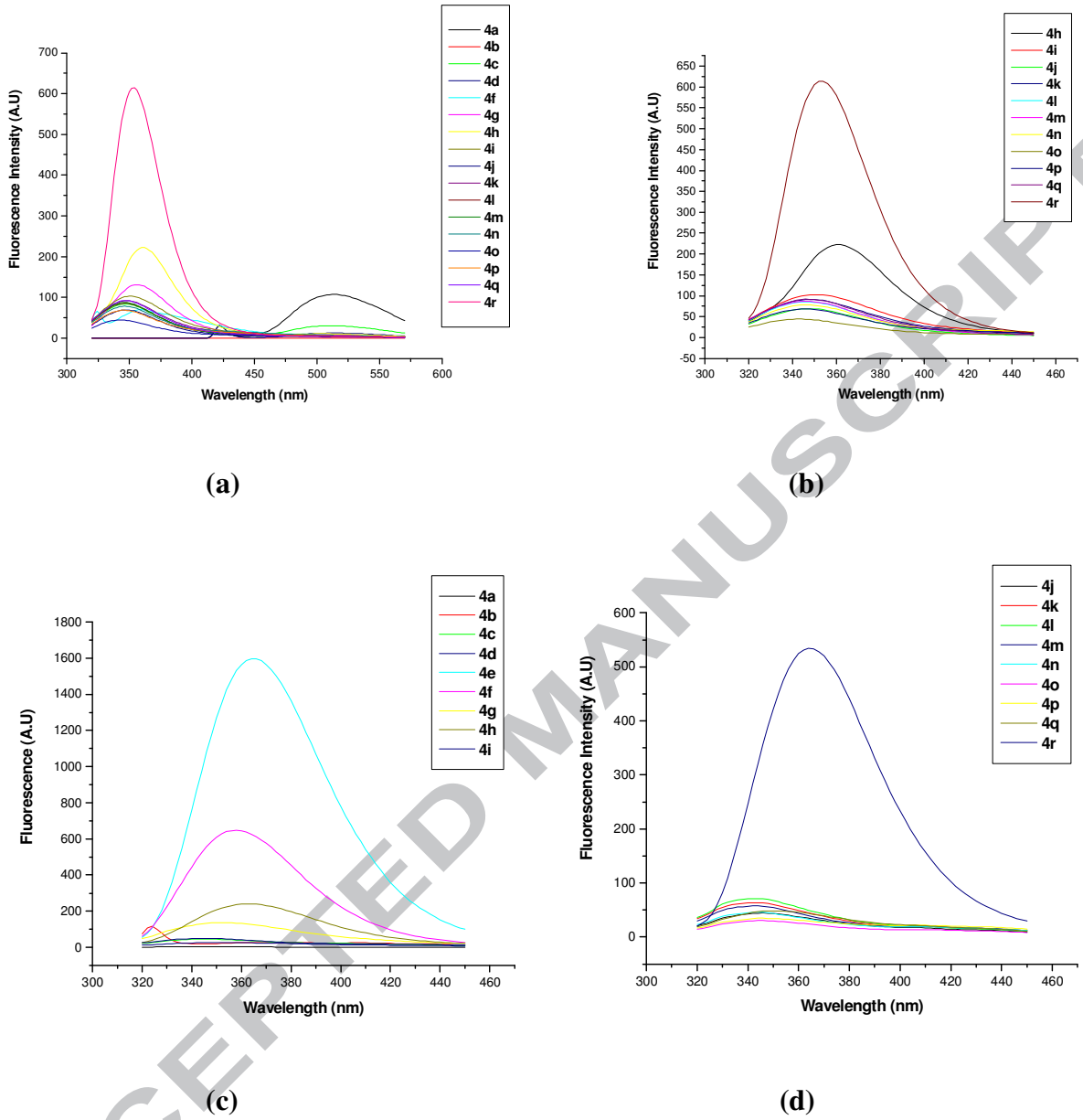


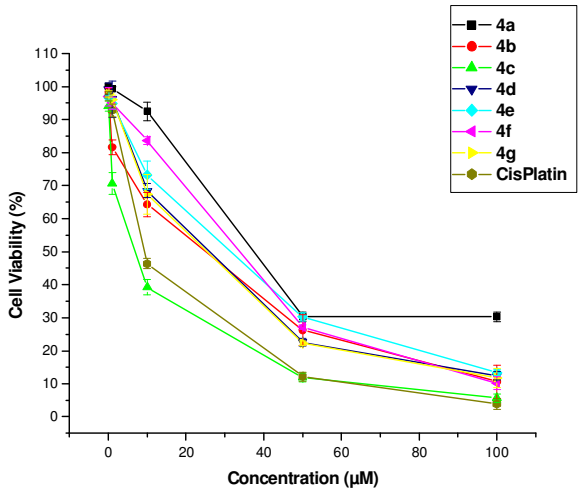
Figure 2. Fluorescence emission spectra of compounds (a) **4a-r** in DMSO; **4e** was not shown as it has no fluorescence (b) **4h-r** in DMSO (c) **4a-i** in CH_2Cl_2 (d) **4j-r** in CH_2Cl_2.

Table 1. Spectroscopic data for compounds **4a-r** at 298 K in DMSO & CH_2Cl_2.

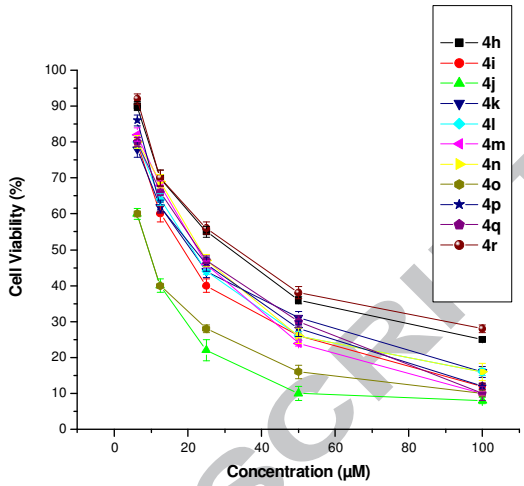
Compounds	DMSO		Dichloromethane	
	λ_{ex}[a] (nm)	λ_{em}[b] (nm)	λ_{ex}[a] (nm)	λ_{em}[b] (nm)
4a	419	514	295	344
4b	618	-	320	372
4c	290	346, 514	295	356
4d	419	514	295	354
4e	498	-	295	378
4f	320	358	295	358
4g	295	356	295	352
4h	290	360	295	364
4i	290	356	295	346
4j	290	346	295	346
4k	290	348	295	344
4l	290	346	295	344
4m	290	346	295	344
4n	290	346	295	344
4o	290	342	295	346
4p	290	346	295	346
4q	290	346	295	350
4r	290	354	295	364

The cytotoxicity of all the synthesized ruthenium complexes **4a-r** (**Table 2**) was evaluated using standard 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) bioassay beside a panel of cell lines such as human Epitheloid Cervix Carcinoma (HeLa) and human breast carcinoma cell line (MCF-7). These cells were treated with compounds (**4a-r**) along with cisplatin & doxorubicin as a standard positive control at concentration 1 to 100 µM for 24 h. All experiments were carried out in triplicates, and IC_{50} was determined from the average of three separate experiments. The inhibition of cell viability of these complexes against HeLa cell line were also evaluated (**Figure 3**). Most of the ruthenium complexes exhibited higher anticancer profile than cisplatin. However, compounds are slightly less potent than doxorubicin. DMSO was used as control where it was not showing any inhibition of cell. In MCF-7 cells, the IC_{50} values for ruthenium pyridinylmethylene scaffolds were in the range of 7–25 µM (**Table 2**). Likewise, in HeLa cells, the IC_{50} values for these complexes were observed in the range of 7-29 µM.

The structure-activity relationship studies revealed that the introduction of electron withdrawing bromo and nitro group at 3rd & 5th position of the pyridine ring (compound **4c** & **4o**) displayed the best cytotoxicity profile. However, cytotoxicity level is slightly reduced after introducing an electron donating methyl group at 6th position of pyridine ring in compound **4c** (compound **4j**). It was also observed that compound **4b** is more cytotoxic than compound **4d** while a methyl group is present at 5th position of pyridine ring in **4b**. These results clearly indicate that substitution of electron withdrawing group at 3rd position of pyridine ring is more important than 5th position. It was concluded that electron donating methyl group at 5th position might not affect the cytotoxicity level of compound **4b** if 3rd position is already occupied by electron withdrawing bromo group. Compounds **4e**, **4f**, **4h** and **4r** exhibited less potency in both the cell lines as electron releasing methyl group is introduced in the pyridine unit of these scaffolds. It was also observed that cytotoxicity levels of **4e** & **4f** are lower than **4h** & **4r**. Hence the cytotoxicity will be reduced more by the substitution of electron releasing group at 3rd and 5th positions in the pyridine ring rather than 4th and 6th position. Compound **4g** also displayed excellent efficacy against both the cell lines. Hydroxyl group present in the 3rd position of the pyridine ring might be responsible for hydrogen bonding with the target protein. Likewise, compounds **4i**, **4k**, and **4l** was also exhibited exceptional cytotoxicity against both the cell line as electronegative chloro or iodo group was present in the pyridine ring. Cytotoxicity of compounds **4m**, **4n**, **4p** and **4q** were maintaining the proper order (**4n<4p<4m<4q**) as well. Highly electron withdrawing bromo & nitro group at the meta position of pyridine ring might be effective for the generation of reactive oxygen species.



(a)



(b)

Figure 3. Dose-dependent drug efficacy studies for (a) **4a-g** and cisplatin (b) **4h-r** on cancer cell lines HeLa

Table 2. Preliminary MTT cytotoxicity screening of synthesized benzothiazole derivatives at 24 h of drug exposure

Compound	IC_{50} (µM)[a]	
	MCF-7	**HeLa**
4a	22.62±0.58	26.32±0.42
4b	08.25±0.76	17.42±0.85
4c	07.95±0.45	07.85±1.16
4d	09.45±0.62	19.6±1.26
4e	25.12±0.72	28.4±0.18
4f	25.42±0.64	29.22±1.68
4g	09.76±0.48	16.20±1.22
4h	24.26±0.78	27.10±1.60
4i	10.76±0.79	20.10±0.50
4j	08.00±0.88	07.96±1.26
4k	09.46±0.98	17.10±0.54
4l	08.66±0.43	16.20±0.66
4m	09.46±0.48	14.30±0.74
4n	10.66±0.88	18.10±1.90
4o	07.76±0.88	07.10±1.28
4p	09.86±0.48	16.22±1.37
4q	08.56±0.82	10.20±0.80
4r	23.86±0.72	27.30±1.26

DMSO	00	00
Cisplatin	9.42±0.52	16.2±0.48
Doxorubicin	5.2±0.28	6.8±0.76

[a]IC_{50} is the concentration at which 50% of cells were undergoing cytotoxic cell death due to synthesized compound treatment.

The toxicity study of most potent anticancer derivative **4o** was carried out using male Sprague Dawley rats. Toxicity profile of test item (**4o**) was compared with doxorubicin (standard) by single intravenous bolus administration in male Sprague Dawley rats (see ESI†). Test group animals were received a single dose injection of test drug at 10mg/kg in normal saline while the control groups were treated with normal saline only. Likewise, reference group animals were received a single dose injection of doxorubicin at 10 mg/kg in normal saline. Afterwards, the animals in all groups were sacrificed for 48 hours. After 48 h of dosing, blood samples were collected from all the rats for estimation of serum Aspartate aminotransferase (AST) and blood glutathione (GSH). After collecting the blood samples, all the animals were sacrificed by exsanguinations under deep isoflurane anesthesia. Heart was removed to estimate the Thiobarbituric Acid Reactive Substances (TBARS) and glutathione (GSH). The blood GSH levels of the doxorubicin (reference group) and test item (compound **4o**) treated animals showed a significant decrease as compared to normal control group ($p < 0.01$). Test item showed a significantly more in blood GSH level as compared to reference ($p < 0.01$). The AST level was elevated significantly in animals treated with doxorubicin as compared to control group. The level was significantly reduced in test group when compared to reference group ($p < 0.01$). Likewise, the level of TBARS was significantly elevated in animals treated with doxorubicin alone as compared to corresponding normal control group. While the animals treated with test item showed significantly low in the concentration of TBARS as compared to reference group. It has also been observed that there was a significant decrease in the level of the heart tissue GSH in

doxorubicin treated group as compared to normal control animals. However animals treated with test item showed significantly more in the concentration of tissue GSH as compared to group II ($p < 0.05$) (**Figure 4**).

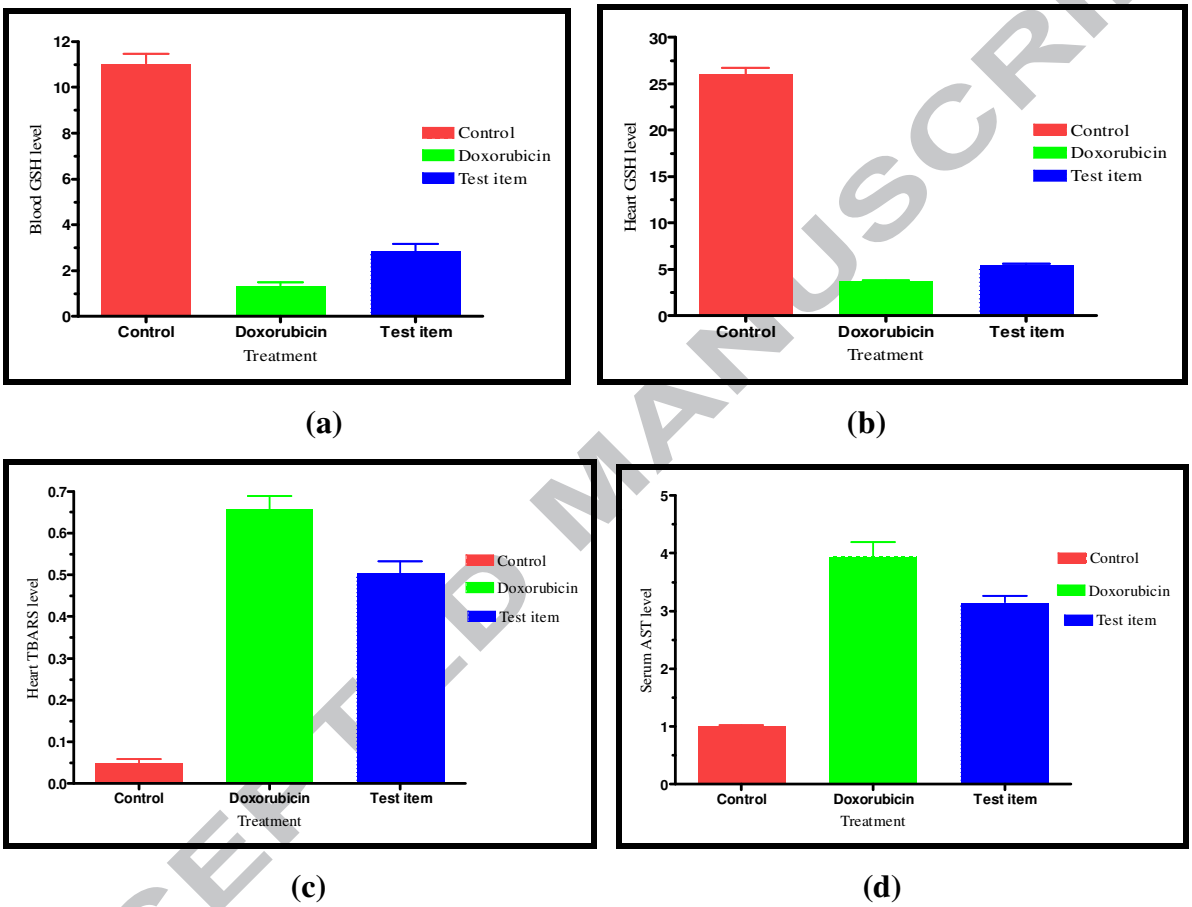


Figure 4. Cardiotoxicity Profile **(a)** blood GSH level **(b)** heart GSH level **(c)** heart TBARS level **(d)** serum AST level

Conclusion

In review, a novel, general and effective procedure for the preparation of Ru-arene N-(pyridin-2-ylmethylene)pyridin-2-amine complexes in a one-pot sequence was established. This technique allows a great compact of synthetic flexibility and offers the opportunity of synthesizing newer organoruthenium coordination for anticancer screening. The significant features of this methodology are that it works with inexpensive and easily available reactants operating in an environment friendly, mild reaction condition with operational simplicity. Most of the compounds exhibited exceptional cytotoxicity against MCF7 and Hela cell lines compared to cisplatin. Those cytotoxic scaffolds also displayed remarkable fluorescence in DMSO and Dichloromethane which might be useful for theranostic application.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/....

[†]Equal Contribution

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29 General procedure for the synthesis of compounds **4a-r**: Ruthenium (II) *p*-cymene Dimer (**3**) (10 mg, 0.016 mmol) was dissolved in 5 ml of methanol in 25 mL RB flask placed on a magnetic stirrer and stirred for 30 min, then Pyridine-2-carboxaldehyde (**1**) & 2-amino pyridine analogues (**2a-r**) (2:2 equivalent with respect to **3**) was added to reaction mixture and continue the stirring for 24 h at room temperature, color change was observed from deep yellow to reddish brown. We performed the TLC in 1% methanol in ethyl acetate which confirms the product. After completion of the reaction we have evaporated the methanol by using rotator evaporator. The crude product was recrystallized from 5% methanol in diethyl ether and orange to red fine crystal was obtained with 95% yield.

[(η6-pcymene)RuCl(κ2-N,N-Pyridin-2-yl-pyridin-2-ylmethylene-amine)]·Cl **(4a)**: **Yield:** 95 %, **Mp:** 146-147 °C, $\mathbf{R_f}$ (1% MeOH in Ethyl Acetate)**:** 0.26, **IR (cm^{-1}):** *υ* 553, 748, 762, 829, 997, 1205, 1360, 1391(aromatic C=C stretch), 1632 (-C=N stretch), 1740, 2338, 2804, 2972 (aromatic C-H stretch), 3044 (aromatic C-H stretch), 3119 (aromatic C-H stretch); **^{1}H NMR** ($CDCl_3$, 400 MHz)**:** δ 1.09 (d, 3H, *J* = 6.8 Hz, Cymene CH_3), 1.30 (d, 3H, *J* = 6.8 Hz, Cymene CH_3), 2.28 (s, 3H, Cymene CH_3), 2.62-2.72 (m, 1H, CH), 5.68 (d, 1H, *J* = 6.8 Hz, Cymene ArH), 5.78 (d, 1H, *J* = 5.6 Hz, Cymene ArH), 6.05 (d, 1H, *J* = 4.8 Hz, Cymene ArH), 6.32 (d, 1H, *J* = 4.8 Hz, Cymene ArH), 7.09-7.14 (m, 1H, ArH), 7.47-7.52 (m, 1H, ArH), 7.91-8.08 (m, 3H, ArH), 8.27 (d, 1H, *J* = 6.0 Hz, ArH), 8.62-8.64 (m, 1H, ArH), 9.04 (brs, 1H, ArCH), 10.13 (s, 1H, imine);

^{13}C NMR ($CDCl_3$, 400 MHz)**:** δ 21.1 (CH_3), 21.7 (CH_3), 28.7 (CH_3), 30.3 (CH), 83.6, 84.3, 85.3, 86.1, 103.7, 105.9, 118.3, 124.7, 129.4, 129.5, 138.5, 138.6, 148.3, 152.4, 156.0, 159.5, 165.6 (-C=N); **^{31}P NMR** ($CDCl_3$, 400 MHz)**:** δ -153.04 (m, PF_6); **^{19}F NMR** ($CDCl_3$, 400 MHz)**:** δ -73.05 (6F, PF_6); ESI-MS (MeOH): m/z: 454 $[M]^+$; HRMS (ESI): m/z calcd for $C_{21}H_{23}N_3ClRu^+$: 454.0624 $[M]^+$; found: 454.0629 $[M]^+$

[(η6-pcymene)RuCl(κ2-N,N-3(3-Bromo-5-methyl-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]·PF_6 **(4b)**: **Yield:** 90 %, **Mp:** 143-145 °C, **R_f** (1% MeOH in Ethyl Acetate)**:** 0.30, **IR (cm^{-1}):** *υ* 553, 675, 742, 770, 779, 829, 1057, 1234, 1302, 1381 (aromatic C=C stretch), 1591(-C=N stretch), 1736, 3119 (aromatic C-H stretch); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ1.05 (d, 3H, *J* = 6.65 Hz, Cymene CH_3), 1.12 (d, 3H, *J* = 6.78 Hz, Cymene CH_3), 2.21 (s, 3H, Cymene CH_3), 2.49 (s, 3H, Cymene CH_3), 2.78-2.85 (m, 1H, CH), 5.45 (brs, 1H, Cymene ArH), 5.72 (d, *J* = 5.52 Hz, 1H, Cymene ArH), 5.82 - 5.91 (m, 2H, Cymene ArH), 7.86 (brs, 1H), 7.99 (brs, 1H, ArH), 8.07 - 8.19 (m, 2H, ArH), 8.47 (brs, 1H, ArH), 8.61 (s, 1H, ArH), 9.51 (brs, 1H, imine); **^{13}C NMR** ($CDCl_3$, 400 MHz)**:** δ 17.8 (CH_3), 20.6 (CH_3), 21.4 (CH_3), 28.7 (CH_3), 30.2 (CH), 83.7, 84.1, 85.3, 85.9, 102.8, 106.3, 110.4, 129.5, 129.8, 135.8, 138.6, 143.0, 147.1, 152.2, 155.6, 155.7, 170.0 (-C=N); **^{31}P NMR** ($CDCl_3$, 400 MHz)**:** δ -153.00 (m, PF_6); **^{19}F NMR** ($CDCl_3$, 400 MHz)**:** δ -72.96 (6F, PF_6); ESI-MS (MeOH): m/z: 546 $[M]^+$, 548 $[M+2]^+$; HRMS (ESI): m/z calcd for $C_{22}H_{24}N_3BrClRu^+$: 545.9886 $[M]^+$; found: 545.9890 $[M]^+$

[(η6-pcymene)RuCl(κ2-N,N-N-(5-bromo-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]·PF_6 **(4d)**: **Yield:** 92 %, **Mp:** 148-150 °C, **R_f** (1% MeOH in Ethyl Acetate)**:** 0.28, **IR (cm^{-1}):** *υ* 555, 692, 717, 739, 770, 822, 1092, 1207, 1306, 1373, 1448 aromatic C=C stretch), 1740 (-C=N stretch), 1970, 3119 (aromatic C-H stretch); **^{1}H NMR** (400 MHz, $CDCl_3$) δ1.07 (d, 3H, *J* = 5.90 Hz, Cymene CH_3), 1.12 (d, 3H, *J* = 6.02 Hz, Cymene CH_3), 2.24 (s, 3H, Cymene CH_3), 2.56-2.62 (m, 1H, CH), 5.53 (brs, 1H, Cymene ArH), 5.68 (brs, 1H, Cymene ArH), 5.74 (brs, 1H, Cymene ArH), 6.0 (brs, 1H, Cymene ArH), 7.87-7.93 (m, 2H, ArH), 8.09-8.16 (m, 3H, ArH), 8.87 (brs, 1H, ArH), 8.67 (brs, 1H, ArH), 9.58 (s, 1H, imine); **^{13}C NMR** (DMSO-*d6*, 400 MHz)**:** δ18.5 (CH_3), 21.7 (CH_3), 30.5 (CH_3), 30.6 (CH), 83.8, 83.9, 86.4, 87.3, 104.8, 105.4, 121.1, 121.4, 129.6, 131.5, 140.0, 142.3, 149.7, 153.9, 156.3, 158.9, 168.8(-C=N); **^{31}P NMR** ($CDCl_3$, 400 MHz)**:** δ -153.02 (m,PF_6); **^{19}F NMR** ($CDCl_3$, 400 MHz)**:**δ -72.87 (6F, PF_6); ESI-MS (MeOH): m/z: 532 $[M]^+$, 534 $[M+2]^+$; HRMS (ESI): m/z calcd for $C_{21}H_{22}N_3BrClRu^+$: 531.9729 $[M]^+$; found: 531.9733 $[M]^+$

[(η6-pcymene)RuCl(κ2-N,N-(4-Methyl-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]·PF_6 **(4f)**: **Yield:** 94 %, **Mp:** 146-147 °C, **R_f** (1% MeOH in Ethyl Acetate)**:** 0.27, **IR (cm^{-1}):** *υ* 519, 555, 692, 710, 739, 771, 822, 1304, 1404 (C=C stretch), 1435 (C=C stretch), 1441 (C=C stretch), 1472 (C=C stretch), 1603, 1632, 1668 (-C=N stretch), 2808, 2934, 2970 (aromatic C-H stretch), 3034 (aromatic C-H stretch), 3042 (aromatic C-H stretch), 3101 (aromatic C-H stretch), 3121, 3323, 3642; **^{1}H NMR** ($CDCl_3$, 400 MHz)**:** δ 1.05-1.09 (m, 3H, Cymene CH_3), 1.29 (d, 3H, J = 7.2 Hz, Cymene CH_3), 2.02 (s, 3H, Cymene CH_3), 2.26 (s, 3H, Me), 2.62-2.69 (m, 1H, CH),5.32 (d, 1H, J = 5.6 Hz, Cymene ArH), 5.54 (d, 1H, J = 5.6 Hz, Cymene ArH), 5.66 (m, 1H, Cymene ArH), 5.76 (brs, 1H, Cymene ArH), 7.50 (m, 1H, ArH), 7.91-8.05 (m, 4H, ArH), 8.61-8.62 (m, 1H, ArH), 9.04 (s, 1H, ArH), 10.1 (s, 1H, imine CH); **^{13}C NMR** ($CDCl_3$, 400 MHz)**:** δ17.8 (CH_3), 20.2 (CH_3), 20.8 (CH_3), 21.1 (CH_3), 30.1 (CH), 81.8, 81.9, 84.0, 85.7, 86.3, 103.55, 104.4, 111.1, 113.5, 118.9, 125.8, 130.6, 135.0, 139.0, 139.4, 150.4, 153.6, 159.8 (-C=N); **^{31}P NMR**

($CDCl_3$, 400 MHz)**:** δ -152.60 (m, PF_6); **^{19}F NMR** ($CDCl_3$, 400 MHz)**:** δ -66.35 (6F, PF_6); ESI-MS (MeOH): m/z: 468 $[M]^+$; HRMS (ESI): m/z calcd for $C_{22}H_{25}N_3ClRu^+$: 468.0781 $[M]^+$; found: 468.0786 $[M]^+$

[(η6-pcymene)RuCl(κ2-N,N-(5-Methyl-pyridin-2-yl)-pyridin-2-ylmethylene-amine)]·PF_6 **(4h): Yield:** 95 %, **Mp:** 140-142°C, **R_f** (1% MeOH in Ethyl Acetate)**:** 0.30, **IR (cm^{-1}):** *v* 515, 555, 692, 739, 768, 820, 1227, 1389, 1470(aromatic C=C stretch), 1589, 1630(-C=N stretch), 2928, 2968(aromatic C-H stretch), 3319; **^{1}H NMR** (DMSO-*d6*, 400 MHz)**:** δ 0.92-0.95 (m, 6H, Cymene CH_3), 2.18 (m, 3H, Cymene CH_3), 2.44 (m, 3H, CH_3), 2.66-2.73 (m, 1H, Cymene CH), 3.15 (s, 3H, Cymene CH_3), 5.71 (d, 1H, *J* = 5.6 Hz, Cymene ArH), 5.82 (d, 1H, *J* = 6 Hz, Cymene ArH), 5.88 (d, 1H, *J* = 6 Hz, Cymene ArH), 6.16 (d, 1H, *J* = 6 Hz, Cymene ArH), 7.78-7.90 (m, 2H, ArH), 7.95 (d, 1H, *J* = 8 Hz, ArH), 8.31-8.51 (m, 3H, ArH), 9.26 (s, 1H, ArH), 9.59 (brs, 1H, imine CH); **^{13}C NMR** (DMSO-*d6*, 400 MHz): δ17.6 (CH_3), 18.5 (CH_3), 21.6 (CH_3), 30.5 (CH_3), 30.6 (CH), 84.0, 84.1, 86.5, 87.1, 119.0, 129.3, 131.0, 133.3, 135.5, 139.9, 146.1, 149.0, 154.1, 156.2, 157.8, 167.5(-C=N); ESI-MS (MeOH): **^{31}P NMR** (DMSO-*d6*, 400 MHz): δ -157.36 (m, PF_6); **^{19}F NMR** (DMSO-*d6*, 400 MHz): δ -71.08 (6F, PF_6); ESI-MS (MeOH): m/z: 468 $[M]^+$; HRMS (ESI): m/z calcd for $C_{22}H_{25}N_3ClRu^+$: 468.0780 $[M]^+$; found: 468.0777 $[M]^+$

Graphical Abstract

Synthesis of novel Anticancer Ruthenium-arene -pyridinylmethylene scaffolds *via* Three-component Reaction

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