Task-specific ionic liquid catalyzed synthesis of novel naphthoquinone–urazole hybrids and evaluation of their antioxidant and in vitro anticancer activity

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Task-specific ionic liquid catalyzed synthesis of novel naphthoquinone–urazole hybrids and evaluation of their antioxidant and *in vitro* anticancer activity†

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We have reported the synthesis of naphthoquinone–urazole hybrids via one pot condensation of 2-hydroxynapthalene-1,4-dione, aldehydes and 4-phenylurazole using task specific ionic liquid (bmim [HSO_4]). All newly synthesized compounds were screened for *in vitro* antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and anticancer activity against human breast (T47D), colon (HCT-15), lung (NCI-H522), liver (HepG-2) and ovary (PA-1) cancer cell lines. All the naphthoquinone–urazole hybrids showed high DPPH radical scavenging activity, comparable to the standard BHT. Compounds **IVf**, **IVh**, **IVi** and **IVj** exhibited very good anticancer activity.

1. Introduction

Cancer is the second most common cause of mortality in the world, and is a health problem in developing as well as developed countries.[1] This disease is now well characterized by unregulated proliferation of cells.[2,3] Chemotherapy is the mainstay of cancer treatment but the choice of available chemotherapeutics is limited mainly due to undesirable side effects. Therefore, development of novel chemotherapeutic agents to inhibit or control cell proliferation is an ongoing research area.

Quinones, including 1,4-naphthoquinones and 1,2-naphthoquinones are ubiquitous in nature and several well-known anticancer drugs used to treat solid tumours (*e.g.* doxorubicin, mitomycin and mitoxantrone) possess a quinonoid structure.[4,5] These compounds have also been identified as privileged structures due to their biological activities and structural properties[6] that have been linked to the stimulation of oxidative stress and alkylation of cellular nucleophiles in cancer cells.[7] Recently, 1,4-naphthoquinones such as menadione[8] juglone[9] plumbagin[10–13] and rhinacanthins[14] have gained importance for their anticancer activity (Fig. 1).

Plumbagin is derived from the plant genus *Plumbago* and has been reported to exhibit cytotoxic properties in various cancer cell lines as well as *in vivo* studies in animal models. Rhinacanthins are isolated from *Rhinacanthus nasutus* and have also been reported for the antitumor activity against P-388, A-549, HT-29, and HL-60 cell lines. On the other hand, urazole (1,2,4-triazolidine-3,5-dione) moiety exhibits a myriad of biological functions such as anticonvulsant[15] antifungal[16] herbicidal[17] hypolipidemic[18] and insecticidal activities.[19] 1-Acyl and 1,2-diacyl-1,2,4-triazolidine-3,5-diones (Fig. 2) are known to be effective cytotoxic agents agents in both murine and human cancer cell lines and were effective *in vivo* in inhibiting Ehrlich ascites carcinoma growth in mice at 8 mg per kg per day.[20] Phenolic and quinone-based compounds are also known to show potent antioxidant activity which is attributed to their redox properties.[21]

Therefore, we considered it worthwhile to fuse naphthoquinone and urazole scaffolds as a single molecular entity in view of enhanced pharmacophoric activity. We believed that such hybrid-heterocycles could be very effective for anticancer activity as hybridization of two different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often showed synergistic effects.[22–26] In view of our experience for the synthesis of hybrid-heterocycles,[27] we focused our efforts on finding an efficient catalyst for the synthesis of naphthoquinone–urazole hybrids using aldehydes as linkers.

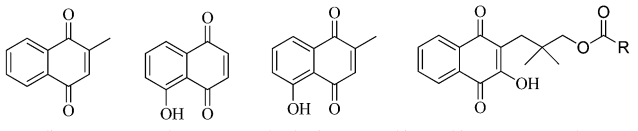

Fig. 1 Naphthoquinone analogs with anticancer activity.

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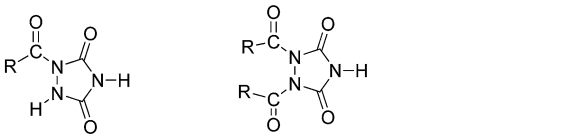


Fig. 2 Urazole analogs with anticancer activity.

2. Results and discussion

2.1 Chemistry

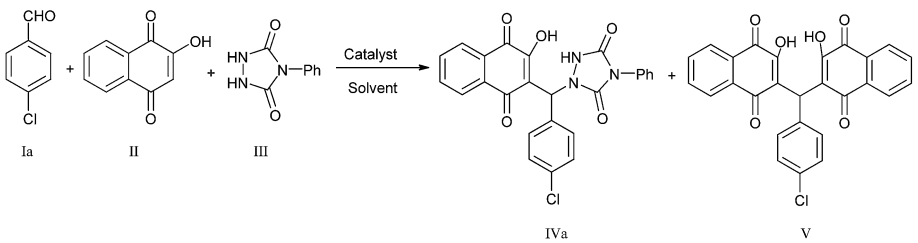
We report herein the synthesis of novel naphthoquinone tethered urazoles namely, 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-diones *via* bmim[HSO_4] catalyzed three-component reaction of aldehydes (**I**), 2-hydroxynapthalene-1,4-dione (**II**) and 4-phenylurazole (**III**). Initially the scavenging effects of the synthesized compounds on DPPH free radical were evaluated. These compounds exhibited very high antioxidant activity and therefore were also evaluated for their anticancer activity. The results of the potency of these compounds against human breast (T47D), colon (HCT-15), lung (NCI-H522), liver (HepG-2) and ovary (PA-1) cancer cell lines are also reported in this paper.

In order to achieve the synthesis of targeted naphthoquinone–urazole hybrids a reaction of 4-chlorobenzaldehyde (**Ia**) (1.0 mmol), 2-hydroxynaphthalene-1,4-dione (**II**) (1.0 mmol) and 4-phenylurazole (**III**) (1.0 mmol) was carried out in the presence of $InCl_3$ as a Lewis acid catalyst in ethanol under reflux (entry 1, Table 1). The product obtained was bis-naphthoquinone identified as 3,3′-((4-chlorophenyl) methylene)bis(2-hydroxynaphthalene-1,4-dione) (**V**) by the reaction of 2-hydroxynaphthalene-1,4-dione and 4-chlorobenzaldehyde while 4-phenylurazole remained unreacted. The above three component condensation was then attempted with other Lewis acids namely, CAN, $CeCl_3$ and La(OTf)$_3$. However, all these Lewis acids gave only bis-naphthoquinone (**V**) (entries 2–4, Table 1). As Lewis acids failed to give the three component condensation product, we screened the application of Bronsted acids for this three-component reaction in ethanol. The reactions carried out using AcOH and HCl as catalysts also yielded only the bis-naphthoquinone (**V**) (entries 5–6, Table 1). However, when the reaction was attempted in presence of *p*-toluenesulfonic acid (*p*-TSA) as a Brønsted acid catalyst in ethanol, it led to the formation of a new product which was

Table 1 Three-component reaction of 4-chlorobenzaladehyde (Ia), 2-hydroxynaphthalene-1,4-dione (II) and 4-phenylurazole (III) under different conditions[a]

Entry	Catalyst	Catalyst (mol%)	Solvent	Temp.	Time (h)	Yield of **IVa** (%)	Yield of **V** (%)
1	$InCl_3$	10	EtOH	Reflux	5	—	85[b]
2	CAN	10	EtOH	Reflux	5	—	61[b]
3	$CeCl_3$	10	EtOH	Reflux	5	—	64[b]
4	La(OTf)$_3$	10	EtOH	Reflux	5	—	55[b]
5	AcOH	10	EtOH	Reflux	5	—	80[b]
6	HCl	10	EtOH	Reflux	5	—	75[b]
7	*p*-TSA	10	EtOH	Reflux	5	28	33
8	H_2SO_4	10	EtOH	Reflux	3	64	13
9	H_2SO_4	10	Water	Reflux	3	49	20
10	H_2SO_4	10	EtOH- Water	Reflux	3	55	15
11	H_2SO_4	10	bmim[BF_4]	60 °C	3	83	—
12	bmim[HSO_4]	10	Neat	60 °C	2	91	—
13	bmim[HSO_4]	5	Neat	60 °C	24	72[c]	—
14	bmim[HSO_4]	20	Neat	60 °C	2	91	—
15	bmim[HSO_4]	10	Neat	r.t.	24	62[c]	—
16	—	—	Neat	60 °C	24	—[d]	—

[a] All the reactions were carried out with 1.0 mmol of **Ia**, 1.0 mmol of **II** and 1.0 mmol of **III**. [b] Yield based on consumption of **II**. [c] Incomplete reaction. [d] No reaction.



Scheme 1

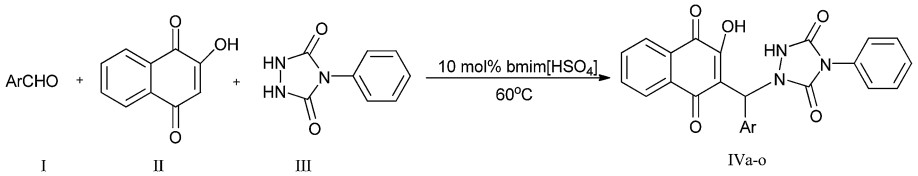
Table 2 Synthesis of 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione derivatives using bmim[HSO_4] as catalyst at 60 °C

Entry	Product	Ar	Time (h)	Yield (%)
1	**IVa**	4-ClC_6H_4	2	91
2	**IVb**	4-$CH_3C_6H_4$	2.5	84
3	**IVc**	4-FC_6H_4	2	89
4	**IVd**	4-$NO_2C_6H_4$	1.5	88
5	**IVe**	4-BrC_6H_4	1.25	90
6	**IVf**	3-BrC_6H_4	1.25	89
7	**IVg**	4-$CH_3OC_6H_4$	2.5	82
8	**IVh**	2-$CH_3C_6H_4$	2	83
9	**IVi**	2-Naphthyl	1.5	89
10	**IVj**	3-$CH_3C_6H_4$	2.5	84
11	**IVk**	3-ClC_6H_4	2.25	90
12	**IVl**	4-(F_3C)C_6H_4	1.5	92
13	**IVm**	2-ClC_6H_4	2	87
14	**IVn**	2,4-$Cl_2C_6H_3$	2	85
15	**IVo**	4-(CN)C_6H_4	2	86

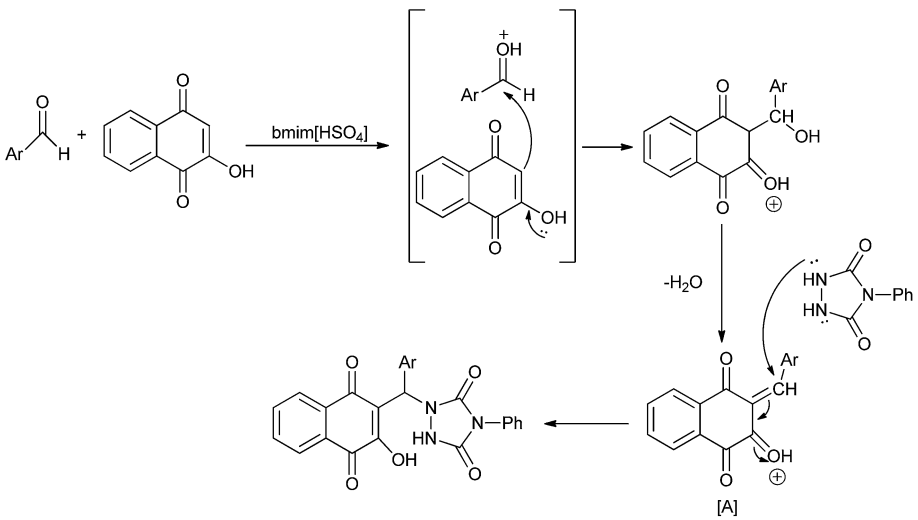
identified as 1-((4-chlorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (**IVa**) in small amount (28%) with bis-naphthoquinone (**V**) as the major product (entry 7, Table 1). The desired product **IVa** was obtained in a moderate yield (64%), when H_2SO_4 was used as the catalyst in ethanol with minor amount of bis-naphthoquinone (**V**) (entry 8, Table 1).

Subsequently, the reactions catalyzed by H_2SO_4 were attempted in water and EtOH–H_2O (1 : 1, v/v) in a similar manner but resulted in inferior yields of the desired product **IVa**, 49% and 55% respectively, along with formation of by-product bis-naphthoquinone (**V**) (entries 9–10, Table 1). To our delight, the formation of by-product, bis-naphthoquinone (**V**), was totally suppressed when the reaction was attempted using H_2SO_4 (10 mol%) as catalyst, in bmim[BF_4] at 60 °C, and resulted into 83% of **IVa** in 3 h (entry 11, Table 1). Therefore, we decided to investigate the reaction using task specific ionic liquid bmim[HSO_4] which could serve as catalyst, medium and could also be recycled. When the reaction of 4-chlorobenzaldehyde (**Ia**) (1.0 mmol), 2-hydroxynaphthalene-1,4-dione (**II**) (1.0 mmol) and 4-phenylurazole (**III**) (1.0 mmol) was carried out in presence of bmim[HSO_4] at 60 °C, both the yield and reaction time improved significantly and afforded the desired product, 1-((4-chlorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (**IVa**) in 91% yield after 2 h (entry 12, Table 1) (Scheme 1).

The amount of bmim[HSO_4] which acts as a catalyst and medium was optimized when the reaction was attempted by varying the catalyst loading amount to 5 and 20 mol% of bmim[HSO_4]. The reaction using 5 mol% of catalyst was incomplete even after 24 h while no such significant improvement in the yield was observed on increasing the loading to 20 mol% (entries 13–14, Table 1). The reaction carried out with 10 mol% bmim[HSO_4] at room temperature was also



Scheme 2



Scheme 3

Table 3 DPPH[a] radical scavenging activity (%) of 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVa–o)

	DPPH scavenging activity (%)				
Comp.	25[b] μM	50[b] μM	100[b] μM	200[b] μM	400[b] μM
IVa	78.79	79.82	80.42	82.20	85.36
IVb	78.63	79.42	82.63	85.96	88.45
IVc	77.59	79.45	80.73	82.60	83.56
IVd	82.30	83.11	85.50	86.55	88.11
IVe	84.03	86.70	86.78	87.13	89.13
IVf	74.42	75.78	78.47	80.56	84.84
IVg	82.54	82.91	84.60	86.47	87.82
IVh	89.85	90.06	91.08	92.40	92.47
IVi	79.72	81.13	82.29	83.74	87.29
IVj	83.51	86.58	89.09	89.46	90.13
IVk	75.43	76.43	78.08	81.39	85.36
IVl	78.11	78.77	82.41	83.80	84.97
IVm	75.84	78.27	80.35	83.93	87.53
IVn	72.18	73.16	74.37	77.23	79.56
IVo	72.13	73.12	75.12	77.96	79.64
BHT[c]	78.58	85.90	86.50	87.20	89.82
2-HNQ	36.52	37.49	38.43	42.08	45.97
4-PU	64.37	88.83	90.98	92.07	93.26

[a] 0.1 mM methanolic solution of DPPH was used for all the experiments. [b] Solution of compounds was prepared in chloroform. [c] Butylated hydroxyl toluene (BHT) was used as a reference. 2-HNQ = 2-hydroxynaphthalene-1,4-dione 4-PU = 4-phenylurazole.

incomplete (entry 15, Table 1). The reaction attempted in absence of catalyst and under solvent free conditions at 60 °C did not yield any product even after 24 h (entry 16, Table 1).

Therefore, a one pot reaction of equimolar amounts of aldehyde (1.0 mmol), 2-hydroxynaphthalene-1,4-dione (1.0 mmol) and 4-phenylurazole (1.0 mmol) using 10 mol% of bmim[HSO_4] as catalyst at 60 °C proved to be the optimum condition. The optimized reaction conditions were then applied to reactions of 2-hydroxynaphthalene-1,4-dione, 4-phenylurazole with different aldehydes having electron withdrawing and electron releasing groups. All the reactions proceeded smoothly to yield the novel 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-diones (**IVa–o**) in high yields (82–92%) (entries 1–15, Table 2) (Scheme 2). Structural assignments have been made on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectra. None of the reaction showed the formation of any bis-condensation product.

A mechanistic rationale portraying the probable sequence of steps is given in Scheme 3. The formation of products **IVa–o** can be rationalized by initial formation of heterodiene [**A**] by Knoevenagel condensation of aldehyde (**I**) and 2-hydroxynaphthalene-1,4-dione (**II**). Subsequent Michael-type addition of urazole (**III**) to heterodiene [A] afforded the corresponding products **IVa–o**.

We also examined the reusability of bmim[HSO_4] by studying the catalytic activity of the recycled ionic liquid for the synthesis of **IVa**. After separation of the product by filtration, the filtrate containing the ionic liquid was rinsed with ether and further vacuumed to dryness at 100 °C for 2 h to remove any moisture. The recovered bmim[HSO_4] was reused directly for the next run. It was found that the ionic liquid can be used for the reactions for upto three subsequent runs without any appreciable loss of efficiency.

Table 4 *In vitro* anticancer activity of 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVa–o)[a]

	Anticancer activity[b] (% growth inhibition) at concentration of 1×10^{-5} M				
Comp.	Breast T47D	Colon HCT-15	Ovary PA-1	Liver HepG2	Lung NCI H-522
IVa	12.58	1.92	57.39	19.17	10.29
IVb	9.62	10.72	56.33	29.87	20.43
IVc	16.83	18.33	0.35	15.41	12.67
IVd	14.54	2.59	51.87	17.69	25.96
IVe	24.77	20.15	44.21	9.48	30.19
IVf	**63.71**	14.67	**59.21**	**61.77**	44.12
IVg	31.75	22.11	34.10	13.92	24.65
IVh	**70.48**	14.30	**57.98**	28.95	24.95
IVi	47.44	**40.27**	**59.27**	1.80	44.36
IVj	**67.56**	29.12	**70.87**	**61.17**	**49.12**
IVk	22.08	24.32	44.46	17.43	28.12
IVl	20.28	29.48	9.60	1.81	30.12
IVm	32.08	34.97	50.10	17.35	44.97
IVn	19.79	14.99	39.60	23.92	24.99
IVo	6.04	23.33	6.10	9.20	13.33
5-FU[c]	20.13	22.87	21.20	21.87	27.54
CYC-PHO[d]	18.10	16.77	34.87	28.67	20.46
CYC-HEXI[e]	26.61	15.18	35.13	18.33	17.48

[a] Bold values represent compounds showing good anticancer activity. [b] Compounds tested in triplicate, data expressed as mean value of three independent experiments. [c] 5-FU 5-fluorouracil. [d] CYC-PHO cyclophosphamide. [e] CYC-HEXI cycloheximide.

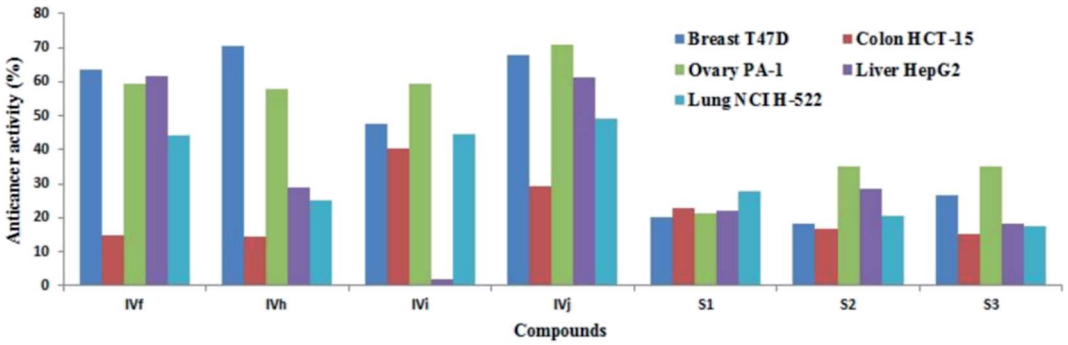


Fig. 3 Graphical representation of anticancer activity of compounds **IVf**, **IVh**, **IVi**, **IVj** and S1 (5-FU), S2 (CYC-PHO), S3 (CYC-HEXI) against five human cancer cell lines.

Table 5 IC_{50} values[a,b] of *in vitro* anticancer activity of active compounds

	IC_{50} (μM)					
Comp.	Breast T47D	Colon HCT-15	Ovary PA-1	Liver HepG2	Lung NCI H-522	Normal cell COS-1
IVf	8 ± 3.9	40 ± 1.08	10.43 ± 2.11	9.67 ± 2.57	16 ± 2.5	75.49 ± 9.12
IVh	5.34 ± 1.16	43 ± 2.1	10.2 ± 2.10	45 ± 7.23	54 ± 9.2	82 ± 8.2
IVi	15 ± 2	20 ± 3.4	9.12 ± 1.11	95.23 ± 12.12	14 ± 3.7	180 ± 4.78
IVj	8.57 ± 1	44 ± 4.9	5.6 ± 2.8	7.89 ± 3.1	10.2 ± 1.3	82.59 ± 3.19
5-FU[c]	51.8 ± 2.34	43.01 ± 1.45	36.5 ± 3.32	29.87 ± 1.82	53.76 ± 3.14	110 ± 8.98
CYC-PHO[d]	70.1 ± 2.32	72.32 ± 4.68	63.12 ± 5.43	51.3 ± 3.59	63.9 ± 3.79	125.43 ± 9.24
CYC-HEXI[e]	65.13 ± 7.31	51.13 ± 3.65	40.6 ± 2.09	57.12 ± 4.65	57.1 ± 5.34	128.31 ± 7.89

[a] 50% growth inhibition as determined by MTT assay (24 h drug exposure). [b] Compounds tested in triplicate, data expressed as mean value ± SD of three independent experiments. [c] 5-FU 5-fluorouracil. [d] CYC-PHO cyclophosphamide. [e] CYC-HEXI cycloheximide.

2.2 Pharmacological results

The naphthoquinone–urazole hybrids (**IVa–o**) were then tested for *in vitro* antioxidant and anticancer activity.

2.2.1 Antioxidant activity. The 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione derivatives (**IVa–o**) were investigated for their antioxidant activity using DPPH assay. The antioxidant activity is represented by their capacities for scavenging DPPH radicals and the results are summarised in Table 3.

It can be inferred from the above data that all the naphthoquinone–urazole hybrids showed potent DPPH radical scavenging activity, comparable to that of standard (BHT) especially **IVe**, **IVh** and **IVj**. Compounds **IVe**, **IVh** and **IVj** which exhibited good antioxidant activity were further studied and their IC_{50} values were found to be 0.64 ± 0.13 μM, 0.37 ± 0.03

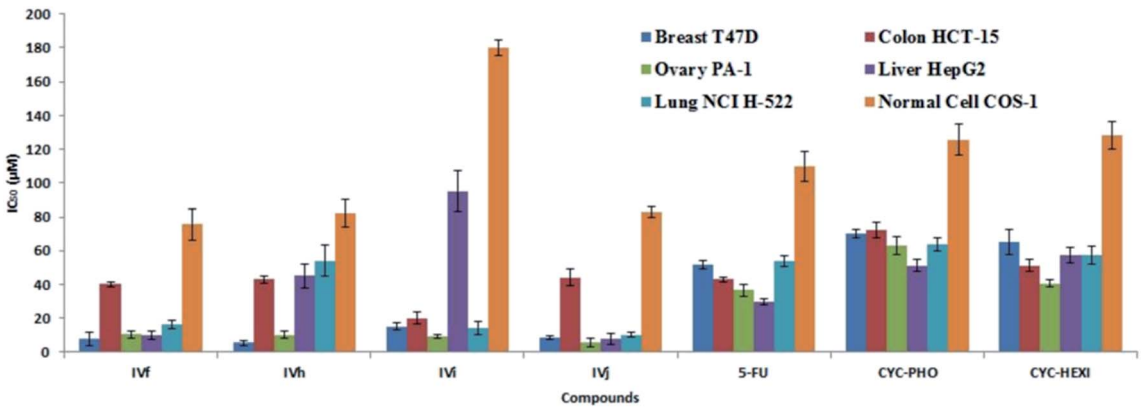


Fig. 4 Graphical representation of IC_{50} values of compounds **IVf**, **IVh**, **IVi**, **IVj** and S1 (5-FU), S2 (CYC-PHO), S3 (CYC-HEXI) against five human cancer cell lines and normal cell.

μM and 0.84 ± 0.12 μM, respectively. The IC_{50} value of standard (BHT) was found to be 10.11 ± 0.50 μM.

2.2.2 *In vitro* anticancer activity. The naphthoquinone–urazole hybrids (**IVa–o**) showed high antioxidant activity comparable to the standard. Therefore, these compounds were screened *in vitro* for anticancer activity[28,29] against five human cancer cell lines *i.e.* breast (T47D), colon (HCT-15), ovary (PA-1), liver (HepG2) and lung (NCI H-522) at a concentration of 1×10^{-5} M and the results are summarized in Table 4. Table 4 indicates that compounds **IVf**, **IVh**, **IVj** exhibited good anticancer activity against breast (T47D) cell line, **IVi** against colon (HCT-15) cell line, **IVf**, **IVh**, **IVi**, **IVj** against ovary (PA-1) cell line, **IVf**, **IVj** against liver (HepG2) cell line and **IVj** against lung (NCIH-522) cell line. Graphical representation of these results is shown in Fig. 3.

Compounds **IVf**, **IVh**, **IVi** and **IVj** which exhibited good anticancer activity against various cell lines were further studied and their IC_{50} values were determined. These IC_{50} values are summarized in Table 5 and Fig. 4. IC_{50} value for all above mentioned compounds for normal cell COS-1 is also reported in Table 5.

3. Conclusion

In summary, we have synthesized a novel series of naphthoquinone–urazole hybrids using ionic liquid bmim[HSO_4] and studied their *in vitro* antioxidant and anticancer activities against different cell lines. The *in vitro* antioxidant and anticancer activity of these compounds revealed that all of the synthesized naphthoquinone–urazole hybrids have a significant activity. 3-Bromo, 2-methyl, 2-naphthyl and 3-methyl derivatives were more active among all the naphthoquinone–urazole hybrids.

4. Experimental protocols

4.1 General

Structures of all of the compounds were identified by their spectroscopic data. Silica gel 60 F254 (precoated aluminium plates) from Merck were used to monitor the reaction progress. Melting points were determined on a Tropical Labequip apparatus and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer FTIR spectrophotometer and the values are expressed as ν_{max} in cm^{-1}. The NMR (^1H and ^{13}C) spectra were recorded on a Jeol JNM ECX-400P at 400 and 100 MHz, respectively. The chemical shift values are recorded on the δ scale and the coupling constants (J) are in Hertz. Mass spectral data were recorded on Waters LCT Micromass spectrometer/Agilent 6520 Q-Tof. Elemental analysis was performed by a Vario EL III Elementar analyzer. Ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate bmim[HSO_4] was synthesized according to a reported procedure.[30]

4.2 General procedure for the synthesis of 1-((aryl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-diones (IVa–o)

A mixture of aldehyde (1.0 mmol), 2-hydroxynaphthalene-1,4-dione (1.0 mmol), 4-phenylurazole (1.0 mmol) and bmim [HSO_4] (10 mol%) was stirred magnetically in an oil-bath maintained at 60 °C for an appropriate time as mentioned in Table 2. After completion of the reaction as monitored by TLC using petroleum ether–ethyl acetate (60 : 40, v/v) as eluent, the reaction mixture was allowed to cool to room temperature and quenched with water (~5 mL). The precipitate formed was collected by filtration at pump and washed well with ethanol to afford the pure naphthoquinone–urazole hybrids. The filtrate was rinsed with ether, concentrated under reduced pressure and dried at 100 °C to recover the ionic liquid for subsequent use.

4.3 DPPH free radical scavenging assay

Methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM) was used as a reagent for the spectrophotometric assay with modifications.[31] Solutions of different concentration of naphthoquinone–urazole hybrids (*i.e.* 25 μM, 50 μM, 100 μM, 200 μM and 400 μM) were prepared using chloroform. 1 mL of 0.1 mM methanolic solution of DPPH was added to 3 mL solution of the compounds and the mixture was shaken vigorously using vortex mixer. Absorbance was read against a blank at 517 nm after incubation of the reaction mixtures for 30 min in dark at room temperature. Butylated hydroxyl toluene (BHT) was used as a reference compound. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. All the tests were carried out in triplicate and the results were averaged.

The IC_{50} was calculated using linear regression analysis.

4.4 *In vitro* cytotoxicity against human cancer cell lines[32]

Human breast (T47D), colon (HCT-15), lung (NCI-H522), liver (HepG-2) and ovary (PA-1) cancer cell lines were obtained from National Center for Cell Science (NCCS), Pune, India. Cells were grown in tissue culture flask in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4 supplemented with 10% fetal bovine serum, 100 μg mL^{-1} streptomycin and 100 units per mL penicillin) in a carbon dioxide incubator (37 °C, 5% CO_2, 90% RH). All cell culture reagents were from GIBCO (Invitrogen, USA). Penicillin, streptomycin, MTT (3-(4, 5-dimethyl-2-thiazolyl) 2,5diphenyl-2*H* tetrazolium bromide), cell culture grade DMSO-d_6, 5 fluorouracil (5-FU), cyclophosphamide and actidione (cycloheximide) were from Himedia (Mumbai, India).

MTT assay was carried out as described previously.[32] In brief, 5×10^3 cells in 200 μL of medium were seeded in 96-well plates (Griener, Germany). Serial dilutions of compound initially ranging from 0–100 μM in DMSO-d_6 were added to the monolayer. The final DMSO-d_6 concentration for all dilutions was 0.1% which was used as vehicle control. The cultures were assayed after 24 h by the addition of 50 μL of 5 mg mL^{-1} MTT and incubating for another 4 h at 37 °C. The MTT-containing

medium was aspirated and 200 μL of DMSO-d_6 (Himedia, Mumbai, India) and 25 μL of Sorensen glycine buffer (0.1 M glycine and 0.1 M NaCl, pH 10.5) were added to lyse the cells and solubilize the water insoluble formazone. Absorbance of the lysates was determined on a Fluostar Optima (BMG Labtech, Germany) microplate reader at 570 nm.

The percentage inhibition was calculated as = 100 − [(mean OD of treated cell × 100)/mean OD of vehicle treated cells (negative control)].

The IC_{50} values were calculated using graph pad prism, version 5.02 software (Graph Pad Software Inc., CA, USA).

5. Spectral data

5.1 1-((4-Chlorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVa)

Yellow solid; M.p.: 234–235 °C; IR (ν_{max} cm^{-1}) (KBr): 3191, 3080, 1761, 1690, 1639; ^{1}H NMR (400 MHz, DMSO) δ = 10.47 (bs, D_2O exch., 1H, NH), 8.03 (t, J = 6.6 Hz, 2H, ArH), 7.88–7.79 (m, 2H, ArH), 7.48–7.45 (m, 4H, ArH), 7.40–7.36 (m, 3H, ArH), 7.31–7.29 (m, 2H, ArH), 6.76 (s, 1H, CH), 3.45 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 183.13, 181.34, 159.09, 152.57, 151.73, 137.14, 134.89, 133.35, 132.11, 131.70, 131.57, 130.28, 128.93, 128.51, 128.26, 128.02, 126.31, 126.14, 125.84, 116.38, 52.83; HRMS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}ClN_3O_5$: 474.0857, found: 474.0851 $[M + H]^+$ (for ^{35}Cl), 476.0836 ($[M + H]^+$ +2) (for ^{37}Cl); anal. calcd for $C_{25}H_{16}ClN_3O_5$: C, 63.37; H, 3.40; N, 8.87%. Found: C, 63.29; H, 3.36; N, 8.81%.

5.2 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-methylphenyl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVb)

Yellow solid; M.p.: 223–224 °C; IR (ν_{max} cm^{-1}) (KBr): 3189, 3060, 1760, 1689, 1643; ^{1}H NMR (400 MHz, DMSO) δ = 10.34 (bs, D_2O exch., 1H, NH), 8.04–8.01 (m, 2H, ArH), 7.89–7.80 (m, 2H, ArH), 7.47 (s, 4H, ArH), 7.40 (s, 1H, ArH), 7.14 (s, 4H, ArH), 6.77 (s, 1H, CH), 3.79 (bs, OH, overlap with DMSO), 2.27 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO): δ = 183.39, 181.27, 158.54, 152.43, 151.72, 136.20, 134.86, 134.63, 133.35, 132.06, 131.75, 130.18, 128.90, 127.93, 126.63, 126.21, 126.12, 125.81, 117.19, 53.21, 20.66; MS (ESI) $[M + H]^+$ calcd for $C_{26}H_{19}N_3O_5$: 454.14, found: 454.26 $[M + H]^+$; anal. calcd for $C_{26}H_{19}N_3O_5$: C, 68.87; H, 4.22; N, 9.27%. Found: C, 68.82; H, 4.19; N, 9.22%.

5.3 1-((4-Fluorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVc)

Yellow solid; M.p.: 230–231 °C; IR (ν_{max} cm^{-1}) (KBr): 3159, 3082, 1762, 1694, 1643; ^{1}H NMR (400 MHz, DMSO) δ = 10.46 (bs, D_2O exch., 1H, NH), 8.04–8.00 (m, 2H, ArH), 7.89–7.79 (m, 2H, ArH), 7.49–7.40 (m, 5H, ArH), 7.34–7.31 (m, 2H, ArH), 7.17–7.13 (m, 2H, ArH), 6.76 (s, 1H, CH), 3.56 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 183.25, 181.34, 160.10, 158.85, 152.54, 151.75, 134.88, 133.91, 133.35, 132.11, 131.73, 130.26, 128.92, 128.83, 127.99, 126.29, 126.13, 125.83, 116.77, 115.18, 114.97, 52.96; HRMS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}FN_3O_5$: 458.1152, found: 458.1147 $[M + H]^+$; anal. calcd for $C_{25}H_{16}FN_3O_5$: C, 65.65; H, 3.53; N, 9.19%. Found: C, 65.58; H, 3.49; N, 9.15%.

5.4 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-nitrophenyl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVd)

Yellow solid; M.p.: 190–192 °C; IR (ν_{max} cm^{-1}) (KBr): 3158, 3075, 1763, 1694, 1640; ^{1}H NMR (400 MHz, DMSO) δ = 10.59 (bs, D_2O exch., 1H, NH), 8.18–8.03 (m, 4H, ArH), 7.87–7.82 (m, 3H, ArH), 7.58–7.41 (m, 6H, ArH), 6.87 (s, 1H, CH), 3.60 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 182.83, 181.28, 159.56, 152.71, 151.84, 146.42, 134.90, 133.36, 132.09, 131.63, 130.33, 128.91, 128.07, 127.61, 126.35, 126.15, 125.86, 123.44, 115.68, 52.90; MS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}N_4O_7$: 485.11, found: 485.17 $[M + H]^+$.

5.5 1-((4-Bromophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVe)

Yellow solid; M.p.: 226-228 °C; IR (ν_{max} cm^{-1}) (KBr): 3191, 3072, 1758, 1688, 1641; ^{1}H NMR (400 MHz, DMSO) δ = 10.46 (bs, D_2O exch., 1H, NH), 8.04–8.01 (m, 2H, ArH), 7.87–7.82 (m, 2H, ArH), 7.52–7.48 (m, 6H, ArH), 7.40 (s, 1H, ArH), 7.25 (d, J = 7.32 Hz, 2H, ArH), 6.74 (s, 1H, CH), 3.74 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 182.91, 181.10, 159.32, 152.52, 151.85, 137.48, 134.84, 133.29, 132.12, 131.68, 131.12, 130.25, 128.88, 127.96, 126.24, 126.09, 125.74, 119.95, 116.32, 52.88; MS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}BrN_3O_5$: 518.04, found: 518.07 $[M + H]^+$.

5.6 1-((3-Bromophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVf)

Yellow solid; M.p.: 223–224 °C; IR (ν_{max} cm^{-1}) (KBr): 3154, 3068, 1762, 1692, 1646; ^{1}H NMR (400 MHz, DMSO) δ = 10.47 (bs, D_2O exch., 1H, NH), 8.04–8.01 (m, 2H, ArH), 7.89–7.80 (m, 2H, ArH), 7.50–7.38 (m, 7H, ArH), 7.32–7.29 (m, 2H, ArH), 6.76 (s, 1H, CH), 3.62 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 183.13, 181.26, 159.07, 152.65, 151.91, 141.02, 134.83, 133.30, 132.12, 131.67, 130.45, 130.33, 129.89, 129.10, 128.94, 128.02, 126.24, 126.11, 125.81, 125.59, 121.68, 116.17, 52.95; HRMS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}BrN_3O_5$: 518.0352, found: 518.0346 $[M + H]^+$ (for ^{79}Br), 520.0314 ($[M + H]^+$ +2) (for ^{81}Br).

5.7 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-methoxyphenyl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVg)

Yellow solid; M.p.: 198–200 °C; IR (ν_{max} cm^{-1}) (KBr): 3192, 3072, 1761, 1690, 1643; ^{1}H NMR (400 MHz, DMSO) δ = 10.29 (bs, D_2O exch., 1H, NH), 8.04 (t, J = 6.6 Hz, 2H, ArH), 7.89–7.79 (m, 2H, ArH), 7.49–7.39 (m, 5H, ArH), 7.20 (d, J = 8.76 Hz, 2H, ArH), 6.88 (d, J = 8.80 Hz, 2H, ArH), 6.73 (s, 1H, CH), 3.72 (s, 3H, OCH_3) 3.65 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 183.52, 181.27, 158.44, 158.22, 152.43, 151.78, 134.89,

133.39, 132.05, 131.76, 130.16, 129.18, 128.92, 128.27, 127.95, 126.24, 126.12, 125.83, 117.50, 113.74, 55.10, 53.32; HRMS (ESI) $[M + H]^+$ calcd for $C_{26}H_{19}N_3O_6$: 470.1352, found: 470.1342 $[M + H]^+$.

5.8 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(2-methylphenyl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVh)

Yellow solid; M.p.: 230–232 °C; IR (ν_{max} cm^{-1}) (KBr): 3180, 3069, 1763, 1690, 1643; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.48$ (bs, D_2O exch., 1H, NH), 8.04–8.00 (m, 2H, ArH), 7.89–7.79 (m, 2H, ArH), 7.49–7.43 (m, 4H, ArH), 7.41–7.37 (m, 1H, ArH), 7.33–7.31 (m, 1H, ArH), 7.18–7.14 (m, 3H, ArH), 6.72 (s, 1H, CH), 3.43 (bs, OH, overlap with DMSO), 2.19 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO): δ = 183.30, 181.09, 158.52, 152.50, 151.23, 135.95, 135.17, 134.91, 133.45, 131.92, 131.73, 130.31, 130.17, 128.96, 127.99, 127.67, 127.40, 126.24, 126.15, 125.83, 116.29, 52.05, 18.88; MS (ESI) $[M + H]^+$ calcd for $C_{26}H_{19}N_3O_5$: 454.14, found: 454.17 $[M + H]^+$; anal. calcd for $C_{26}H_{19}N_3O_5$: C, 68.87; H, 4.22; N, 9.27%. Found: C, 68.84; H, 4.19; N, 9.23%.

5.9 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-(naphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVi)

Yellow solid; M.p.: 218–220 °C; IR (ν_{max} cm^{-1}) (KBr): 3151, 3068, 1758, 1694, 1647; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.47$ (bs, D_2O exch., 1H, NH), 8.06 (t, $J = 8.04$ Hz, 2H, ArH), 7.88–7.81 (m, 6H, ArH), 7.52–7.41 (m, 8H, ArH), 6.96 (s, 1H, CH), 3.96 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): δ = 183.31, 181.36, 158.93, 153.40, 152.53, 151.83, 135.49, 134.86, 133.32, 132.84, 132.19, 131.78, 130.29, 128.91, 128.81, 127.95, 127.87, 127.43, 126.29, 126.14, 125.91, 125.83, 125.17, 124.96, 116.84, 53.66; MS (ESI) $[M - H]^+$ calcd for $C_{29}H_{19}N_3O_5$: 488.12, found: 488.16 $[M - H]^+$.

5.10 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(3-methylphenyl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVj)

Yellow solid; M.p.: 226–228 °C; IR (ν_{max} cm^{-1}) (KBr): 3185, 3066, 1762, 1690, 1646; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.33$ (bs, D_2O exch., 1H, NH), 8.05 (t, $J = 6.60$ Hz, 2H, ArH), 7.89–7.80 (m, 2H, ArH), 7.52–7.38 (m, 5H, ArH), 7.23–7.20 (m, 1H, ArH), 7.08–7.05 (m, 3H, ArH), 6.79 (s, 1H, CH), 3.74 (bs, OH, overlap with DMSO), 2.27 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO): $\delta = 183.32, 181.32$, 158.72, 152.47, 151.73, 137.77, 137.39, 134.85, 133.32, 132.09, 131.74, 130.23, 128.93, 128.24, 127.95, 127.72, 127.06, 126.22, 126.14, 125.81, 123.68, 117.05, 53.23, 21.14; MS (ESI) $[M + H]^+$ calcd for $C_{26}H_{19}N_3O_5$: 454.14, found: 454.16 $[M + H]^+$.

5.11 1-((3-Chlorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVk)

Yellow solid; M.p.: 222–224 °C; IR (ν_{max} cm^{-1}) (KBr): 3152, 3065, 1761, 1693, 1640; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.47$ (bs, D_2O exch., 1H, NH), 8.04–8.01 (m, 2H, ArH), 7.89–7.82 (m, 2H, ArH), 7.48 (s, 4H, ArH), 7.40–7.31 (m, 4H, ArH), 7.24 (d, $J = 6.60$ Hz, 1H, ArH), 6.77 (s, 1H, CH), 3.64 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): $\delta = 183.07, 181.22, 159.01, 152.63$, 151.86, 140.76, 134.80, 133.27, 133.04, 132.09, 131.67, 130.31, 130.12, 128.90, 127.96, 126.96, 126.33, 126.20, 126.09, 125.79, 125.17, 116.18, 52.96; MS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}ClN_3O_5$: 474.08, found: 474.01 $[M + H]^+$.

5.12 1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)(4-(trifluoromethyl)phenyl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVl)

Yellow solid; M.p.: 212–213 °C; IR (ν_{max} cm^{-1}) (KBr): 3187, 3074, 1762, 1691, 1648; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.56$ (bs, D_2O exch., 1H, NH), 8.05–8.01 (m, 2H, ArH), 7.96–7.92 (m, 2H, ArH), 7.90–7.86 (m, 1H, ArH), 7.84–7.79 (m, 1H, ArH), 7.77–7.74 (m, 1H, ArH), 7.70–7.68 (m, 2H, ArH), 7.52–7.39 (m, 4H, ArH), 6.85 (s, 1H, CH), 3.55 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): $\delta = 183.01, 181.23, 159.24, 152.67, 151.79, 143.26$, 134.87, 133.35, 132.07, 131.66, 130.30, 128.91, 128.03, 127.64, 127.15, 126.30, 126.15, 125.83, 125.17, 115.96, 52.88; HRMS (ESI) $[M + H]^+$ calcd for $C_{26}H_{16}F_3N_3O_5$: 508.1120, found: 508.1114 $[M + H]^+$.

5.13 1-((2-Chlorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVm)

Yellow solid; M.p.: 217–218 °C; IR (ν_{max} cm^{-1}) (KBr): 3149, 3067, 1764, 1689, 1640; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.58$ (bs, D_2O exch., 1H, NH), 8.05 (t, $J = 6.6$ Hz, 2H, ArH), 7.90–7.81 (m, 2H, ArH), 7.50–7.37 (m, 7H, ArH), 7.35–7.31 (m, 2H, ArH), 6.79 (s, 1H, CH), 3.44 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): $\delta = 183.13, 181.12, 153.39, 152.49, 151.30$, 135.53, 134.95, 133.35, 131.97, 131.65, 131.56, 130.23, 129.58, 129.19, 128.93, 128.79, 127.98, 127.10, 126.18, 126.10, 125.87, 115.56, 52.18; MS (ESI) $[M + H]^+$ calcd for $C_{25}H_{16}ClN_3O_5$: 474.08, found: 496.03 $[M + Na]^+$ (for ^{35}Cl), 498.04 ($[M + Na]^+$+2]) (for ^{37}Cl).

5.14 1-((2,4-Dichlorophenyl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-4-phenyl-1,2,4-triazolidine-3,5-dione (IVn)

Yellow solid; M.p.: 213–215 °C; IR (ν_{max} cm^{-1}) (KBr): 3183, 3070, 1759, 1686, 1644; ^{1}H NMR (400 MHz, DMSO) $\delta = 10.64$ (bs, D_2O exch., 1H, NH), 8.05 (t, $J = 8.08$ Hz, 2H, ArH), 7.92–7.81 (m, 2H, ArH), 7.60 (s, 1H, ArH), 7.55–7.51 (m, 1H, ArH), 7.49–7.40 (m, 6H, ArH), 6.73 (s, 1H, CH), 3.45 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): $\delta = 182.78, 181.00, 158.75, 152.55$, 151.52, 135.01, 134.91, 133.49, 132.84, 132.38, 131.80, 131.57, 130.99, 130.20, 128.92, 128.64, 128.05, 127.27, 126.25, 126.14, 125.93, 115.04, 52.00; HRMS (ESI) $[M + H]^+$ calcd for $C_{25}H_{15}Cl_2N_3O_5$: 508.0467, found: 508.0462 $[M + H]^+$ (for ^{35}Cl), 510.0435 ($[M + H]^+$ +2) (for ^{35}Cl and ^{37}Cl), 512.0410 ($[M + H]^+$ +4) (for ^{37}Cl).

5.15 4-((3,5-Dioxo-4-phenyl-1,2,4-triazolidin-1-yl)(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)benzonitrile (IVo)

Yellow solid; yield: 86%; M.p.: 218–220 °C; IR (ν_{max} cm^{-1}) (KBr): 3220, 3064, 2230, 1779, 1702, 1640; ^1H NMR (400 MHz, DMSO) $\delta = 10.64$ (bs, D_2O exch., 1H, NH), 8.04–8.01 (m, 2H, ArH), 7.94 (s, 1H, ArH), 7.89–7.85 (m, 1H, ArH), 7.82 (d, $J = 8.08$ Hz, 2H, ArH), 7.50–7.49 (m, 4H, ArH), 7.45–7.39 (m, 1H, ArH), 7.33 (d, $J = 8.76$ Hz, 2H, ArH), 6.85 (s, 1H, CH), 3.42 (bs, OH, overlap with DMSO); ^{13}C NMR (100 MHz, DMSO): $\delta = 185.05$, 180.37, 154.95, 152.67, 152.40, 140.17, 136.02, 134.14, 132.70, 132.33, 131.05, 129.19, 128.93, 128.67, 128.38, 127.51, 126.87, 125.43, 117.96, 112.65, 53.99; MS (ESI) $[M + H]^+$ calcd for $C_{26}H_{16}N_4O_5$: 465.12, found: 465.13 $[M + H]^+$.

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