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SYNTHESIS AND CYTOTOXICITY ACTIVITY OF SOME NOVEL HYDRAZIDE, PYRAZOLE, ISOXAZOLE, PYRIMIDINE AND FUSED PYRAN-2-ONE DERIVATIVES

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Abstract – The reaction of 2-cyano-3-(dimethylamino)-*N*-((2-methoxynaphthalen-1-yl)methylene)acrylohydrazide (**2**) with some nitrogen nucleophiles, phenols and compounds, having an active methylene group to obtain polyfunctionally substituted azoles (**3-5**), azine (**6**), fused azines (**7-10**) and fused pyran-2-one derivatives (**11-17**). All the synthesized products were confirmed by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and MS data. The antitumor evaluation of the newly synthesized products against four human tumor cells lines namely hepatocellular carcinoma (liver) HepG-2, colorectal carcinoma (colon) HCT-116, mammary gland (breast) MCF-7 and epidermoid carcinoma (larynx) Hep-2 was investigated. Compound **4** exhibited superior *in vitro* antitumor activity in the 3-cell lines assay.

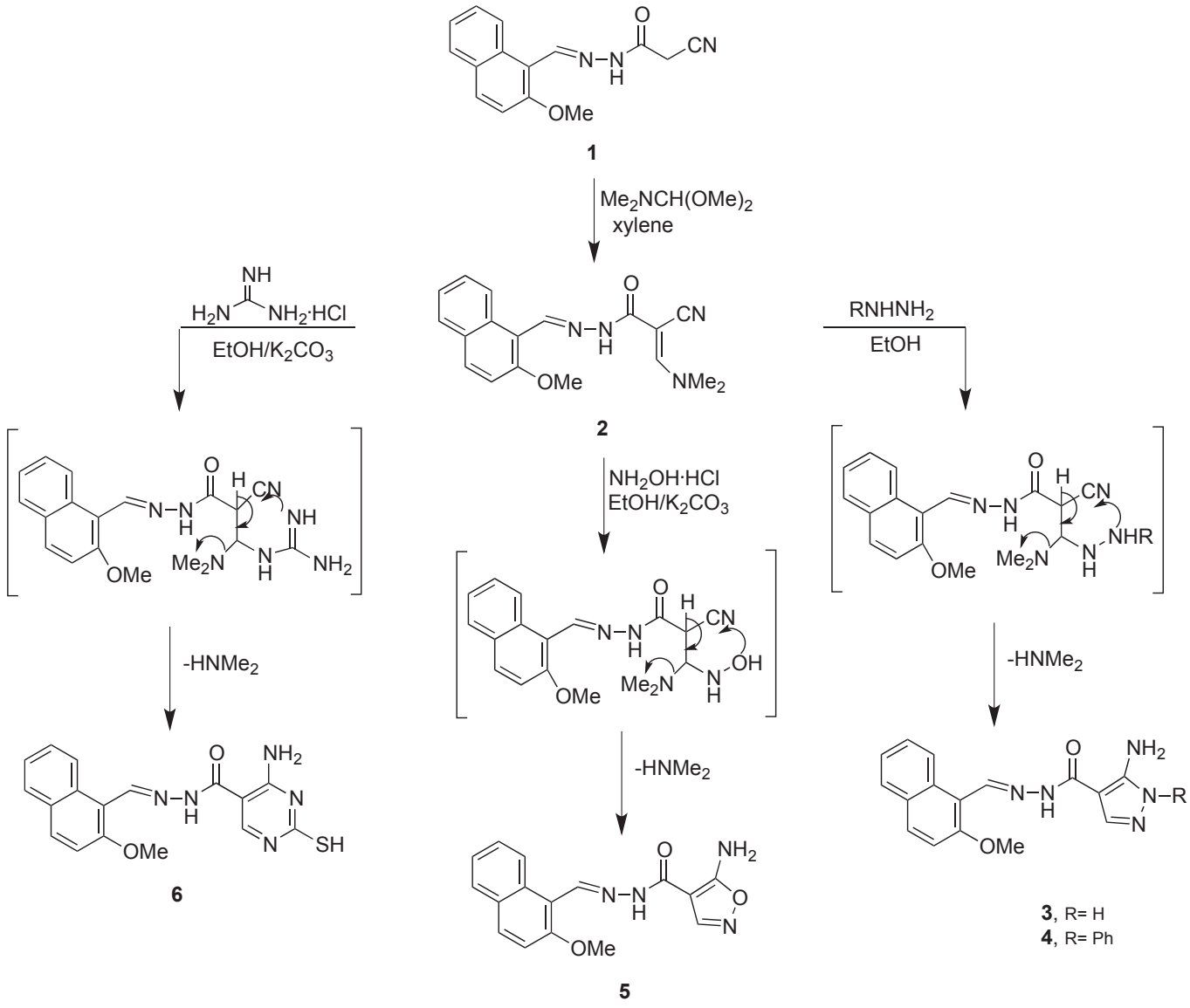
Enaminonitriles are important intermediates for the synthesis of heterocyclic compounds having various biological activities.[1-7] It's investigated and exhibited a wide range of bioactivities including antitumor,[8] antimicrobial,[9] antiviral,[10] analgesic, and anti-inflammatory drugs[11] have been reported. On the other hand, hydrazones possessing an azomethine group (-CONH-N=CH-) constituted an important class of compounds for new drug development[12] and showed a lot of bioactivity such as antimicrobial,[13] antitubercular,[14,15] anticonvulsant,[16] analgesic,[17] anti-inflammatory,[18,19] antiplatelet aggregation,[20] anticancer,[21,22] antifungal,[23] antiviral,[24] antibacterial[25] and antimalarial[26] activities. These facts have prompted us for the development of new hydrazones and evaluated for their antitumor activities. In continuation of our interest an ongoing program aiming at finding new structural leads with potential

chemotherapeutic activities;[27-30] it was an excuse to synthesize some novel polysubstituted heterocycles linked to bicyclic naphthalene skeleton through a hydrazone linkage of potential interest. We report herein a facial synthesis of the highly versatile, hitherto unreported 2-cyano-3-(dimethylamino)-*N*-((2-methoxynaphthalen-1-yl)methylene)acrylohydrazide (2) and the results of its utility as a building block for the synthesis of the title compounds that would produce anticancer activity.

The target compounds are depicted in Schemes 1-4. The starting compound, 2-cyano-3-(dimethylamino)-*N*-((2-methoxynaphthalen-1-yl)methylene)acrylohydrazide (2) was prepared by refluxing an equimolar amounts of 2-cyano-*N*-((2-methoxynaphthalen-1-yl)methylene)acetohydrazide (1)[31] and dimethylformamide dimethyl acetal (DMF-DMA) in dry xylene. The assignment of structure 2 was supported by elemental analysis and spectral data. For example, its ^{1}H-NMR spectrum revealed two singlet signals at δ 3.27 and 3.33 ppm due to NMe_2 group, also, a singlet signals at δ 4.07 and 8.17 ppm due to OMe and vinylic protons, respectively. Further, the mass spectrum of 2 showed the molecular ion peak at *m/z* = 322 (M^+, 86.17), which matches with its molecular formula $C_{18}H_{18}N_4O_2$.

The reactivity of enaminonitrile 2 towards some nitrogen nucleophiles was investigated. Thus, when enaminonitrile 2 was reacted with hydrazine hydrate and phenylhydrazine in refluxing ethanol furnished the pyrazole derivatives 3 and 4, respectively. Structures of compounds 3 and 4 were established on the basis of elemental analysis and spectral data. The IR spectra of pyrazoles 3 and 4 were free of nitrile function and showed absorption bands for NH_2 at 3450-3320 cm^{-1}. The ^{1}H-NMR spectra of 3 and 4 displayed a singlet signal at δ 8.35 ppm, which represents the C_3 proton of the pyrazole ring. Also, the mass spectra showed the molecular ion peaks at *m/z* = 309 (M^+, 5.25%), and 385 (M^+, 7.49%), respectively, which are in agreement with their molecular formulas.

Similarly, the enaminonitrile **2** reacts with hydroxylamine hydrochloride in refluxing ethanol containing anhydrous potassium carbonate afforded isoxazole derivative **5**. The assignment of structure **5** was supported by elemental analysis and spectral data. The IR spectrum displayed stretching vibration bands at 3410, 3379, 3201 and 1635 cm^{-1} corresponding to NH_2, NH and CO groups, respectively. Its ^{1}H-NMR spectrum revealed the presence of signals at δ 4.05, 6.13, 9.04 and 11.74 ppm assignable for OCH_3, CH=N and NH protons, respectively. The mass spectrum showed the molecular ion peak at *m/z* = 310 (M^+, 36.58%) corresponding to the molecular formula ($C_{16}H_{14}N_4O_3$).



Scheme 1

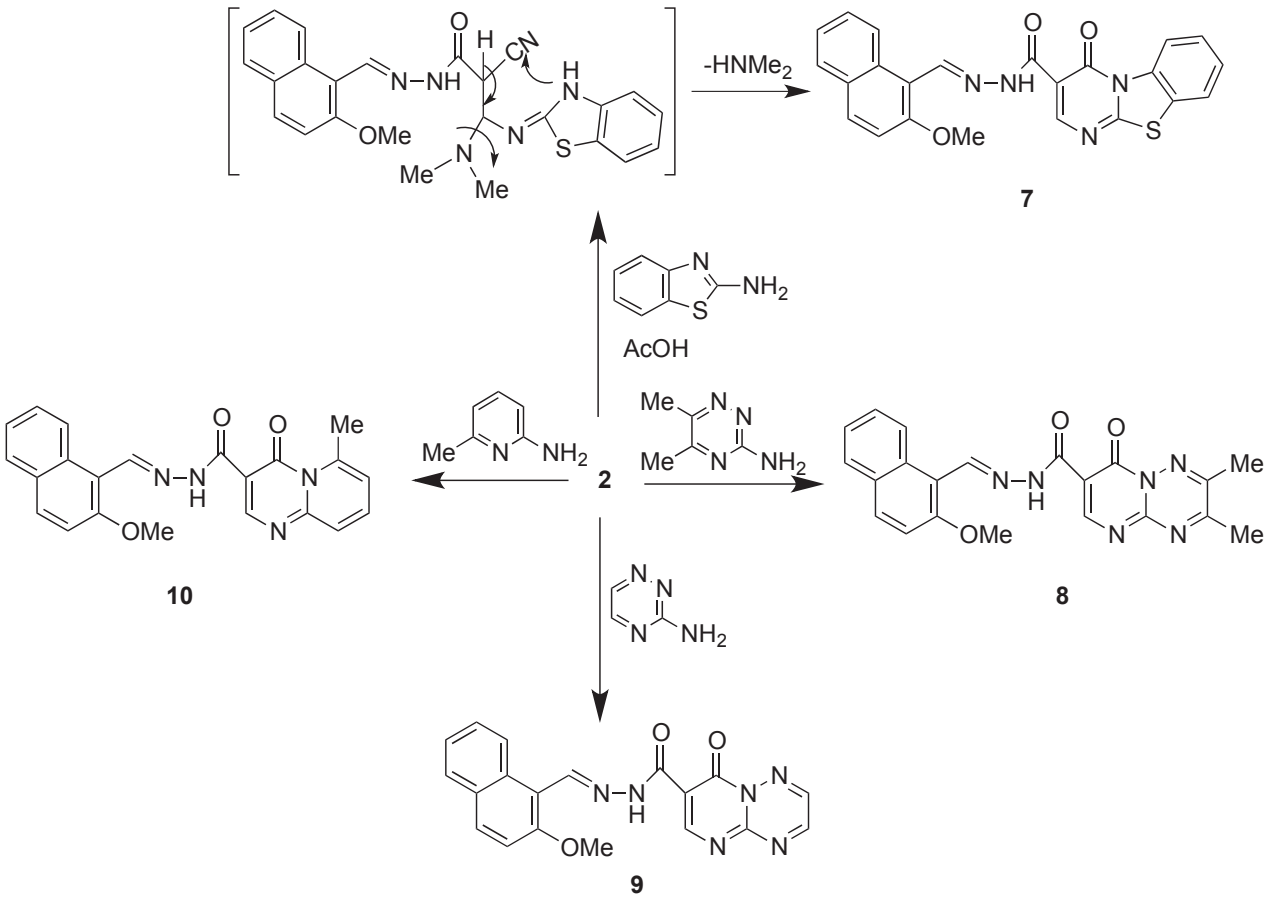
We have also investigated the reactivity of enaminonitrile **2** towards guanidine. Thus, when **2** was treated with guanidine hydrochloride in refluxing ethanol containing anhydrous potassium carbonate afforded 2,4-diamino-*N*-((2-methoxynaphthalen-1-yl)methylene)pyrimidine-5-carbohydrazide (**6**). The assignment of structure **6** was supported by elemental analysis and spectral data. The mass spectrum of **6** showed the molecular ion peak at *m/z* = 336 (M^+, 10.37) corresponding to a molecular formula $C_{17}H_{16}N_6O_2$.

Compounds **3-6** are assumed to be formed *via* addition of *N*-nucleophiles to the activated ethylenic double bond of enaminonitrile **2**, followed by intramolecular cyclization and elimination of a dimethylamine molecule to give target compounds **3-6**, is in line with the reported results of the reaction of enaminonitrile with nucleophiles under basic conditions.[32]

On the other hand, we investigated the reactivity of enaminonitrile **2** towards some *N,N*-binucleophiles to give benzothiazolo[3,2-*a*]pyrimidine (**7**), 2,3-dimethylpyrimido[1,2-*b*][1,2,4]triazine (**8**),

pyrimido[1,2-*b*][1,2,4]triazine (**9**) and pyrido[1,2-*a*]pyrimidine (**10**), which expected pharmaceutical interest. Thus, reaction of enaminonitrile **2** with equimolar amounts of heterocyclic amines namely 2-aminobenzothiazole, 3-amino-5,6-dimethyl triazole, 3-aminotriazole and 2-amino-6-methylpyridine in refluxing acetic acid afforded the corresponding fused pyrimidine derivatives **7-10** (Scheme 2).

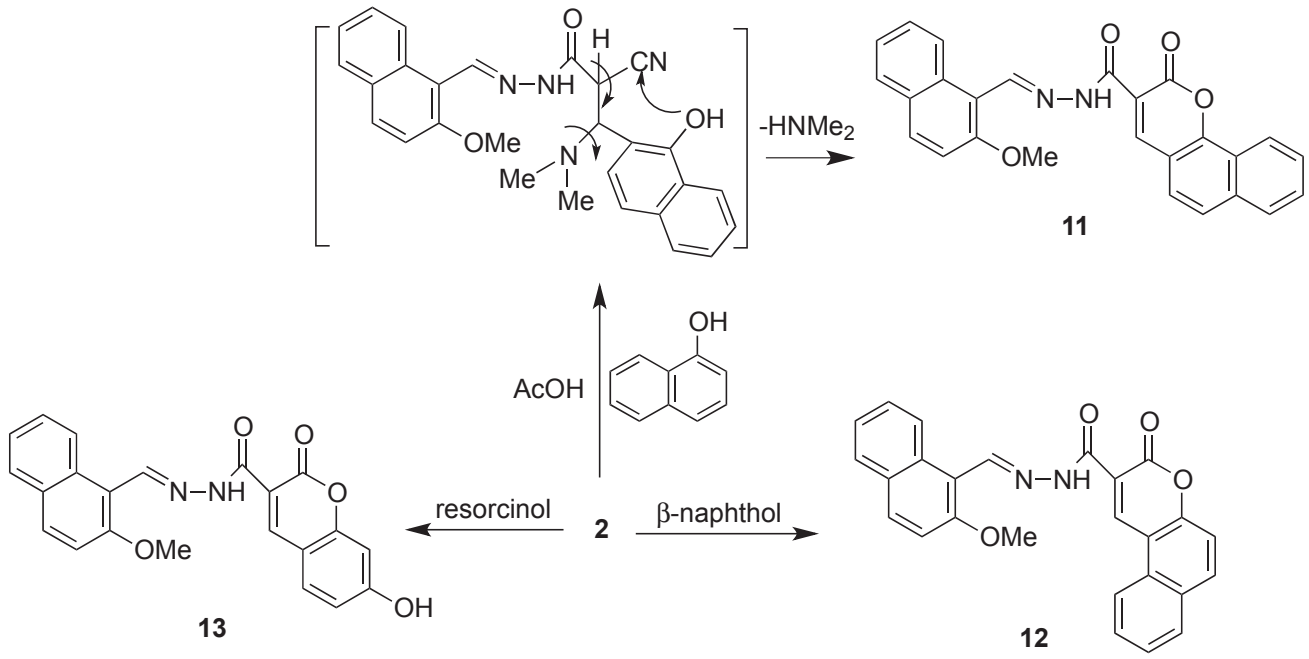
The structures **7-10** were established by the correct elemental analysis and compatible spectroscopic data. These products were confirmed by the disappearance of a cyano absorption band around 2186 cm^{-1} in their IR spectra, and the presence of a characteristic singlet signal was due to the C_4 proton of pyrimidine ring at δ 8.96 ppm in their ^{1}H-NMR spectra.



Scheme 2

Next, we studied the reactivity of the enaminonitrile **2** towards some phenols, having an active methylene group to obtain fused pyran-2-one linked to indanone and pyrimidine moieties through a carbohydrazide linkage has been investigated. Thus, treatment of enaminonitrile **2** with phenolic group such as α-naphthol, β-naphthol and resorcinol under acidic medium gave the corresponding chromene derivatives **11-13**. Analytical and spectral data for the later compounds were in agreement with the proposed structures.

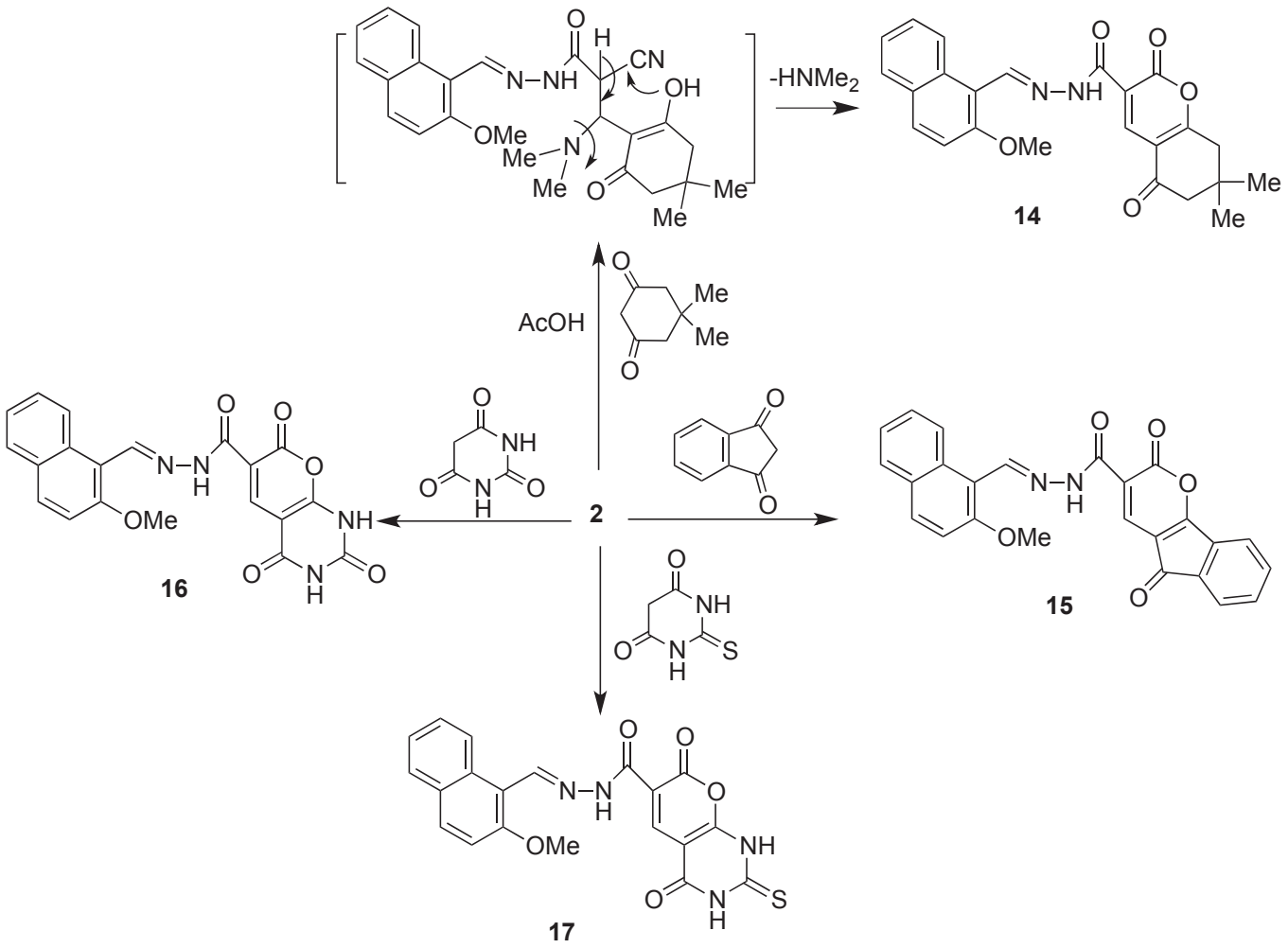
Generally, the IR spectra showed the absence of absorption band of cyano group and presence of the absorption band at 1713-1724 cm^{-1}due to lactone carbonyl group, (see experimental).



Scheme 3

Moreover, refluxing enaminonitrile **2** with active methylene compound such as dimedone in acetic acid afforded *N*-((2-methoxynaphthalen-1-yl)methylene)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydro-2*H*-chromene-3-carbohydrazide (**14**). The assignment of structure **14** was supported by elemental analysis and spectral data. Its IR spectrum displayed stretching vibration bands at 3437 cm^{-1} corresponding to NH group, in addition to, the stretching vibration of three carbonyl groups at 1719-1642 cm^{-1}. Its ^{1}H-NMR spectrum revealed the presence of singlet signals at δ 1.09, 1.91, 2.33 ppm assignable for two CH_2 and CH_3 protons. Also, the mass spectrum showed the molecular ion peak at *m/z* = 418 corresponding to the molecular formula $C_{24}H_{22}N_2O_5$.

Furthermore, *N*-((2-methoxynaphthalen-1-yl)methylene)-2,5-dioxo-2,5-dihydroindeno[1,2-*b*]pyran-3-carbohydrazide (15) could be achieved by reaction of enaminonitrile 2 with 1,3-indandione, in acetic acid under reflux. The assignment of structure 15 was based on analytical and spectral data. The IR spectrum displayed stretching vibration bands at 3435, 1705 and 1637 cm^{-1} corresponding to NH and CO groups, respectively. Its ^{1}H-NMR spectrum revealed the presence of signals at δ 3.84, 8.54, 9.06 and 11.07 ppm assignable for OCH_3, C_4 of coumarin ring, CH=N and NH protons, respectively. The mass spectrum showed the molecular ion peak at *m/z* = 424 (M^+, 20.68%) corresponding to the molecular formula $C_{25}H_{16}N_2O_5$.



Scheme 4

Finally, the reaction of enaminonitrile **2** with either barbituric acid or thiobarbituric acid in acetic acid afforded the corresponding pyrano[2,3-*d*]pyrimidine derivatives **16, 17**, respectively. The structures **16, 17** were established by the correct elemental analysis and compatible spectroscopic data (see experimental). Similar to the proposed reaction mechanism in scheme 3, compounds **11-17** were formed.

CYTOTOXICITY ACTIVITY

All the newly synthesized compounds, ten analogs were selected to be evaluated for their *in vitro* anticancer effect *via* the standard MTT method,[33-35] against a panel of four human tumor cell lines namely; hepatocellular carcinoma (liver) HepG-2, colorectal carcinoma (colon) HCT-116, mammary gland (breast) MCF-7 and epidermoid carcinoma (larynx) Hep-2. The cell lines were obtained from ATCC via the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil (5-Fu)

was used as a standard anticancer drug for comparison. The results of cytotoxic activity are reported in **Table 1**.

Table 1. Cytotoxicity (IC_{50}) of tested compounds on different cell lines

Compounds	*In- vitro* Cytotoxicity IC_{50} (μg/mL)			
	HePG2	HCT-116	MCF-7	Hep-2
5-FU	2.35	4.98	6.56	3.51
2	2.60	5.22	6.87	3.69
3	3.17	6.07	7.65	4.51
4	2.16	4.86	5.90	3.65
5	2.81	5.47	7.10	4.14
6	2.90	5.66	6.95	4.51
7	4.34	6.95	8.55	5.50
8	5.21	7.89	9.02	6.41
9	4.71	7.20	8.76	5.92
10	5.42	8.41	9.12	6.87
11	2.63	5.65	8.85	3.93
12	2.71	5.81	6.91	4.05
13	2.41	5.05	6.24	3.56
14	2.51	5.05	6.82	3.84
15	3.72	6.74	8.29	5.22
16	2.98	5.74	7.45	4.54
17	2.95	5.67	7.30	4.24

IC50 (μmol/L): (1-10) very strong, 11-25 (strong), 26-50 (moderate), 51-100 (very weak), 200 (non-cytotoxicity), 5-Fu = 5-Fluorouracil.

The obtained results revealed that compound 4 are more potent and efficacious than 5-fluorouracil as reference drug towards the three tested human tumor cell lines. As for activity against hepatocellular carcinoma HepG-2, the highest cytotoxic activity was displayed by compounds 2, 4, 13 and 14 which showed the percentage viability IC_{50} at 2.60, 2.16, 2.41 and 2.51 μg/mL, respectively.

Colorectal carcinoma (colon) HCT-116 cell line showed the highest sensitivity towards the tested compounds, as its growth was found to be initiated by five compounds. The best activity was demonstrated by compounds 2, 4, 5, 13 and 14 which have IC_{50} at 5.22, 4.86, 5.47, 5.02 and 5.02 μg/mL, respectively. On the other hand, mammary gland (breast) MCF-7 cell line showed highest sensitivity towards the tested

compounds, as its growth was found to be initiated by five compounds. The best activity was demonstrated by compounds 2, 4, 5, 13 and 14 which have IC_{50} at 6.87, 5.90, 7.10, 6.24 and 6.82 μg/mL, respectively. Further interpretation of the results revealed that compounds 2, 4, 5, 13 and 14 showed high anticancer activity against breast cancer MCF-7 with percentage inhibition range of 3.56-4.24 μg/mL.

STRUCTURE ACTIVITY RELATIONSHIP

By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following structure activity relationships (SAR) were postulated.

- The presence of a basic skeleton carbohydrazide is necessary for the broad spectrum of cytotoxic activity towards different cell lines (HepG-2, HCT-116, MCF-7 and Hep-2).
- Introducing of *N*-phenyl pyrazole ring to carbohydrazide derivative increases the cytotoxic activity.
- Transformation of carbohydrazide derivative to pyrimido[1,2-*b*][1,2,4]triazine reduces the antitumor activity, may be due its bulky size and fused heterocyclic system.
- The pyran ring containing hydroxyl group increases the cytotoxic activity.
- Introducing of isoxazole ring to carbohydrazide derivative increases the activity towards HCT-116 and MCF-7.

In conclusion, the objective of the present study was to synthesize and investigate the anticancer activity of some novel carbohydrazide with the hope of discovering new structure leads to serving as anticancer agents. The results of the anticancer screening revealed that compounds 2, 4, 5, 13 and 14 exhibited the highest *in vitro* cytotoxic activity when compared with the other tested compounds and 5-fluorouracil as a reference drug. In particular compound 4 proved to the most active member in this study with special effective against the human HepG-2, HCT-116 and MCF-7.

EXPERIMENTAL

Melting points Melting points were measured with a Gallenkamp apparatus are uncorrected. IR spectra were recorded KBr discs on a Mattson 5000 FTIR spectrophotometer at Microanalytical Unit, Faculty of Science, Mansoura University. The ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker WP AC 300 (75 MHz) in DMSO-d_6 as solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δppm. Mass spectra were determined on Finnigan Incos 500 (70 eV). Elemental analyses were carried out at the Microanalytical Centre, Faculty of Science, Cairo University, Egypt. The results were found to be in good agreement with the calculated values.

Synthesis of 2-cyano-3-(dimethylamino)-*N*-(2-methoxynaphthalen-1-yl)methylene)acrylohydrazide (2). A mixture of compound **1** (2.67 g, 0.01 mol) and dimethylformamide dimethyl acetal (1.32 mL, 0.01 mol) in dry xylene (25 mL) was refluxed for 4 h, then left to cool at room temperature. The orange

precipitate product was filtered off and recrystallized from toluene to give compound **2**; Orange crystals; yield (82%); mp 178-180 ºC (toluene); IR (KBr): ν/cm^{-1}= 3245 (NH), 2186 (CN), 1666 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.27 (s, 3H, NCH_3), 3.33 (s, 3H, NCH_3), 4.07 (s, 3H, OCH_3), 7.44-8.14 (m, 6H, Ar-H), 8.17 (s, 1H, CH=C), 9.05 (s, 1H, CH=N), 11.74 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 43.5 (2C), 55.5, 96.0, 104.3, 115.5, 119.5 (2C), 123.0, 126.2, 128.0, 129.4, 132.2, 133.2, 143.0, 153, 155.5, 172.0; MS (EI, 70 eV) *m/z* (%) = 322 (M^+, 86.17), 292 (57.45), 251 (72.21), 221 (67.02), 199 (58.51), 184 (55.32), 169 (100.00). Anal. Calcd for $C_{18}H_{18}N_4O_2$ (322.37): C, 67.07; H, 5.63; N, 17.38%. Found: C, 67.02; H, 5.57; N, 17.29%.

General procedure for the reaction of enaminonitrile 2 with *N*-nucleophiles. To a solution of enaminonitrile **2** (0.64 g, 2 mmol) in EtOH (20 mL), hydrazine hydrate (80%, 0.2 mL) or phenylhydrazine (0.2 mL, 2 mmol) was added. The reaction mixture was refluxed for 8 h, and then cooled. The solid product so formed was filtered off, washed with EtOH, dried and recrystallised from a mixture of DMF/EtOH (1:2) to give compounds **3** and **4**.

5-Amino-*N*-((2-methoxynaphthalen-1-yl)methylene)-1*H*-pyrazole-4-carbohydrazide (3). Yellow powder; yield (76%); mp 265-266 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3450, 3369 (NH_2), 3275, 3125 (2NH), 1661 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.06 (s, 3H, OCH_3), 6.81 (s, 2H, NH_2), 7.44-8.16 (m, 6H, Ar-H), 8.35 (s, 1H, pyrazole H_3), 9.02 (s, 1H, CH=N), 9.50, 11.74 (s, 2H, 2NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.5, 104.5, 114.0, 119.1 (2C), 123.4, 126.3, 128.2, 129.5, 130.6, 132.4, 133.5, 134.6, 143.0, 153, 168.0; MS (EI, 70 eV) *m/z* (%) = 309 (M^+, 5.25), 265 (4.01), 225 (4.01), 184 (7.43), 169 (14.27), 139 (3.24). Anal. Calcd for $C_{16}H_{15}N_5O_2$ (309.33): C, 62.13; H, 4.89; N, 22.64%. Found: C, 62.02; H, 4.83; N, 22.54%.

5-Amino-*N*-((2-methoxynaphthalen-1-yl)methylene)-1-phenyl-1*H*-pyrazole-4-carbohydrazide (4). Yellow powder; yield (65%); mp 198-200 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3428, 3320 (NH_2), 3228 (NH), 1662 (C=O), 1624 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.06 (s, 3H, OCH_3), 6.74 (s, 2H, NH_2), 7.52-8.21 (m, 6H, Ar-H), 8.34 (s, 1H, pyrazole H_3), 9.02 (s, 1H, CH=N), 11.72 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.6, 104.5, 119.2 (2C), 123.4 (3C), 126.4 (2C), 128.0, 129.5 (3C), 132.0, 133.0, 139.0, 140.0, 143.3, 150, 153.5 (2C), 168.0; MS (EI, 70 eV) *m/z* (%) = 385 (M^+, 7.49), 351 (5.95), 276 (10.56), 228 (5.85), 213 (8.10), 185 (29.95). Anal. Calcd for $C_{22}H_{19}N_5O_2$ (385.43): C, 68.56; H, 4.97; N, 18.17%. Found: C, 68.52; H, 4.93; N, 18.12%.

Synthesis of 5-amino-*N*-((2-methoxynaphthalen-1-yl)methylene)isoxazole-4-carbohydrazide (5). A mixture of enaminonitrile **2** (0.64 g, 2 mmol) and hydroxylamine hydrochloride (0.18 g, 2.3 mmol) in EtOH (20 mL) containing anhydrous potassium carbonate (0.55 g, 4 mmol) was heated under refluxed for 6 h, then allowed to cool at room temperature and diluted with ice cold water (30 mL). The solid product so formed was filtered off, washed with water and recrystallised from EtOH to afford compound **5**; Yellow

crystals; yield (74%); mp 198-200 ºC (EtOH); IR (KBr): ν/cm^{-1}= 3410, 3379 (NH_2), 3201 (NH), 1661 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.05 (s, 3H, OCH_3), 6.13 (s, 2H, NH_2), 7.49-8.15 (m, 6H, Ar-H), 8.31 (s, 1H, isoxazole H_3), 9.04 (s, 1H, CH=N), 11.74 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.5, 104.5, 108.4, 119.0 (2C), 124, 126.6, 128.0, 129.0, 132.0, 133.0, 143.0, 153.3, 160.0, 168.0, 171.0; MS (EI, 70 eV) *m/z* (%) = 310 (M^+, 36.58), 296 (22.18), 280 (28.79), 227 (22.57), 186 (28.40), 153 (35.80). Anal. Calcd for $C_{16}H_{14}N_4O_3$ (310.31): C, 61.93; H, 4.55; N, 16.06%. Found: C, 61.89; H, 4.47; N, 16.09%.

Synthesis of 2,4-diamino-*N*-((2-methoxynaphthalen-1-yl)methylene)pyrimidine-5-carbohydrazide (6). A mixture of enaminonitrile **2** (0.64 g, 2 mmol) and guanidine hydrochloride (0.22 g, 2.3 mmol) in EtOH (20 mL) containing anhydrous potassium carbonate (0.55 g, 4 mmol) was heated under refluxed for 8 h. The reaction mixture was allowed to cool to room temperature and diluted with ice-cold water (30 mL) containing few drops with HCl. The solid product so formed was filtered off, washed with water and recrystallised from EtOH to afford compound **6**; Yellow crystals; yield (86%); mp 190-192 ºC (EtOH); IR (KBr): ν/cm^{-1}= 3396, 3340 ($2NH_2$), 3197 (NH), 1660 (C=O); ^{1}H-NMR (300 MHz, DMSO- d_6) δ (ppm): 3.98 (s, 3H, OCH_3), 6.61, 6.92 (s, 4H, $2NH_2$), 7.39-8.25 (m, 6H, Ar-H), 8.56 (s, 1H, pyrimidine H_6), 9.05 (s, 1H, CH=N), 11.53 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO- d_6) δ (ppm): 55.5, 104.2, 108.2, 119.0 (2C), 124.3, 126.6, 128.0, 129.5, 132.4, 133.0, 143.2, 153.4, 155.0, 162.0, 163.0, 168.0; MS (EI, 70 eV) *m/z* (%) = 336 (M^+, 10.37), 287 (11.81), 214 (11.29), 183 (54.72), 153 (35.43), 137 (100.00). Anal. Calcd for $C_{17}H_{16}N_6O_2$ (336.35): C, 60.71; H, 4.79; N, 24.99%. Found: C, 60.63; H, 4.70; N, 24.93%.

General procedure for the reaction of enaminonitrile 2 with *N,N*-binucleophiles. To a solution of enaminonitrile **2** (0.64 g, 2 mmol) in acetic acid (10 mL), an equimolar amount of the appropriate heterocyclic amines (2-aminobenzothiazole, 5,6-dimethyl-3-amino-1,2,4-triazine , 3-amino-1,2,4-triazine and 2-amino-6-methylpyridine) was added and the mixture was heated under reflux for 10-14 h, then evaporated in vacuo. The residue was triturated with EtOH and the was collected by filtration, dried well, and recrystallized from a mixture of EtOH/DMF (1:1) to give compounds **7-10**.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-4-oxo-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine-3-carbo-hydrazide (7).** Brown powder; yield (65%); mp 286-288 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3437 (NH), 1666 (2C=O), 1627 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.98 (s, 3H, OCH_3), 7.60-8.68 (m, 10H, Ar-H), 8.96 (s, 1H, pyrimidine-H_4), 9.09 (s, 1H, CH=N), 11.06 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.5, 104.3, 119.2 (2C), 122.4, 124.2 (2C), 125.5, 126.4 (2C), 128.3, 129.4 (2C), 130.5, 132.0, 133.0, 136.0, 143.3, 153.2, 155.0, 158.0, 163.5, 168.0; MS (EI, 70 eV) *m/z* (%) = 428 (M^+, 35.68), 307 (24.07), 281 (21.58), 230 (26.14), 184 (24.90), 153 (26.97), 126 (34.85). Anal. Calcd for $C_{23}H_{16}N_4O_3S$ (428.47): C, 64.47; H, 3.76; N, 13.08%. Found: C, 64.40; H, 3.68; N, 13.12%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-2,3-dimethyl-8-oxo-8*H*-pyrimido[1,2-*b*][1,2,4]triazine-7-carbohydrazide (8).** Brown powder; yield (68%); mp > 300 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3403 (NH), 1662 (2C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.49 (s, 6H, CH_3), 3.97 (s, 3H, OCH_3), 7.09-8.22 (m, 6H, Ar-H), 8.97 (s, 1H, pyrimidine-H_4), 9.08 (s, 1H, CH=N), 11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 18.5, 23.2, 55.5, 104.5, 119.5 (2C), 124.4, 126.2, 128.0, 129.4 (2C), 132.2, 133.3, 143.5, 145.3, 153.4 (2C), 155.0, 160.0, 166.3, 168.2; MS (EI, 70 eV) *m/z* (%) = 402 (M^+, 11.20), 386 (10.16), 296 (11.33), 199 (7.55), 180 (11.33), 153 (6.77). Anal. Calcd for $C_{21}H_{18}N_6O_3$ (402.41): C, 62.68; H, 4.51; N, 20.88%. Found: C, 64.31; H, 4.46; N, 20.82%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-8-oxo-8*H*-pyrimido[1,2-*b*][1,2,4]triazine-7-carbohydrazide (9)**. Brown powder; yield (74%); mp > 300 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3432 (NH), 1663 (2C=O), 1621 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.05 (s, 3H, OCH_3), 7.61-8.65 (m, 8H, Ar-H), 8.96 (s, 1H, pyrimidine-H_4), 9.09 (s, 1H, CH=N), 11.08 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.3, 104.2, 119.0 (2C), 124.3, 126.5, 128.0, 129.2 (2C), 132.3, 133.1, 136.0, 143.3, 150.1, 153.4 (2C), 155.0, 166.0, 168.0; MS (EI, 70 eV) *m/z* (%) = 374 (M^+, 26.62), 327 (23.05), 280 (20.13), 225 (26.30), 186 (34.42), 173 (27.27). Anal. Calcd for $C_{19}H_{14}N_6O_3$ (374.36): C, 60.96; H, 3.77; N, 22.45%. Found: C, 60.86; H, 3.70; N, 22.39%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-6-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbohydrazide (10).** Reddish brown powder; yield (77%); mp > 300 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3430 (NH), 1663 (2C=O), 1627(C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.45 (s, 3H, CH_3), 4.06 (s, 3H, OCH_3), 6.65-8.35 (m, 9H, Ar-H), 8.97 (s, 1H, pyrimidine-H_4), 9.06 (s, 1H, CH=N), 11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 23.5, 55.5, 104.4, 118.2, 119.2 (2C), 124.1, 125.0, 126.2 , 128.0, 129.3 (2C), 132.1, 133.3, 134.0, 143.2 (2C), 150.0, 153.1, 155.0, 160.0, 168.0; MS (EI, 70 eV) *m/z* (%) = 386 (M^+, 42.92), 340 (24.66), 290 (36.99), 265 (47.03), 215 (33.79), 180 (38.36), 155 (39.73). Anal. Calcd for $C_{22}H_{18}N_4O_3$ (386.41): C, 68.38; H, 4.70; N, 14.50%. Found: C, 68.33; H, 3.62; N, 14.55%.

General procedure for the reaction of enaminonitrile 2 with phenols and compounds, have an active methylene group. To a solution of enaminonitrile **2** (0.64 g, 2 mmol) in acetic acid (10 mL), and α-naphthol or β-naphthol (0.28 g, 2 mmol) or resorcinol (0.22 g, 2 mmol) or dimedone (0.28 g, 2 mmol) or 1,3-indandione (0.29 g, 2 mmol) or barbituric acid (0.25 g, 2 mmol) or thiobarbituric acid (0.28 g, 2 mmol) was refluxed for 10 h. The obtained solid product was collected while it hot by filtration and recrystallized from a mixture of EtOH/DMF (1:1) to give compounds **11-17**.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-2-oxo-2*H*-benzo[*h*]chromene-3-carbohydrazide (11).** Brown powder; yield (73%); mp > 300 ºC (EtOH-DMF) ; IR (KBr): ν/cm^{-1}= 3423 (NH), 1716, 1662 (2C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.04 (s, 3H, OCH_3), 7.20-8.14 (m, 12H, Ar-H), 8.54 (s, 1H, coumarin-H_4), 9.09 (s, 1H, CH=N), 11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz,

DMSO-d_6) δ (ppm): 55.4, 104.5, 114.2, 117.5, 119.6 (3C), 120.5 (3C), 122.0, 124.3, 126.4 (3C), 127.5 , 128.2, 129.4, 132.2, 133.1, 134.3, 143.2, 153.3, 155.5, 160.0, 168.0; MS (EI, 70 eV) *m/z* (%) = 422 (M^+, 54.30), 350 (40.00), 285 (22.90), 214 (28.60), 185 (25.70), 167 (31.40), 126 (34.30). Anal. Calcd for $C_{26}H_{18}N_2O_4$ (422.44): C, 73.93; H, 4.30; N, 6.63%. Found: C, 73.87; H, 4.27; N, 6.55%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-3-oxo-3*H*-benzo[*f*]chromene-2-carbohydrazide (12).** Red powder; yield (78%); mp > 300 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3418 (NH), 1713, 1660 (2C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.06 (s, 3H, OCH_3), 7.29-8.16 (m, 12H, Ar-H), 8.54 (s, 1H, coumarin-H_4), 9.09 (s, 1H, CH=N),11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.6, 104.2, 114.1, 115.5, 117.2, 119.3 (2C), 122.2, 123.5, 124.2, 126.6 (2C), 128.5 (3C), 129.4, 130.2 (2C), 132.1, 133.1, 143.3, 144.2, 150.3, 153.5, 160.2, 168.4; MS (EI, 70 eV) *m/z* (%) = 422 (M^+, 23.14), 391 (18.57), 346 (22.57), 320 (24.00), 213 (18.86), 199 (18.57), 186 (15.71). Anal. Calcd for $C_{26}H_{18}N_2O_4$ (422.44): C, 73.92; H, 4.30; N, 6.63%. Found: C, 73.83; H, 4.22; N, 6.65%.

7-Hydroxy-*N*′-((2-methoxynaphthalen-1-yl)methylene)-2-oxo-2*H*-chromene-3-carbohydrazide (13). Orange powder; yield (81%); mp > 300 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3453 (OH), 3204 (NH), 1724, 1663 (2C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.06 (s, 3H, OCH_3), 7.12-8.18 (m, 9H, Ar-H), 8.54 (s, 1H, coumarin-H_4), 9.09 (s, 1H, CH=N), 10.23 (s, 1H, OH), 11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.5, 104.0 (2C), 112.3, 114.1, 117.2, 119.4 (3C), 124.2, 126.5, 128.5, 129.2, 130.1, 132.2, 133.1, 143.2, 153.3, 156.0, 158.5, 160.0, 168.2; MS (EI, 70 eV) *m/z* (%) = 388 (M^+, 33.50), 370 (26.60), 353 (36.45), 313 (42.86), 226 (28.08), 199 (38.42), 181 (32.02). Anal. Calcd for $C_{22}H_{16}N_2O_5$ (388.38): C, 68.04; H, 4.15; N, 7.21%. Found: C, 67.97; H, 4.09; N, 7.18%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydro-2*H*-chromene 3-carbohydrazide (14).** Buff powder; yield (85%); mp 165-166 °C (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3437 (NH), 1719-1642 (3C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 1.09 (s, 6H, $2CH_3$), 1.91 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 4.07 (s, 3H, OCH_3), 7.26-7.95 (m, 6H, Ar-H), 8.54 (s, 1H, coumarin-H_4), 9.02 (s, 1H, CH=N), 11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 28.1 (2C), 33.2, 42.5, 43.6, 55.6, 104.5, 114.2, 119.3 (2C), 123.1, 124.6, 126.7, 128.3, 129.5, 132.2, 133.3, 143.2, 153.4, 154.5, 156.4, 160.3, 168.5, 186.2; MS (EI, 70 eV) *m/z* (%) = 418 (M^+, 17.93), 387 (13.64), 374 (14.39), 357 (16.41), 266 (14.65), 252 (23.23), 185 (15.66), 173 (17.93), 126 (25.25). Anal. Calcd for $C_{24}H_{22}N_2O_5$ (418.45): C, 68.89; H, 5.30; N, 6.69%. Found: C, 68.79; H, 5.21; N, 6.63%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-2,5-dioxo-2,5-dihydroindeno[1,2-*b*]pyran-3-carbohydrazide (15).** Red powder; yield (82%); mp > 300 °C (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3435 (NH), 1705, 1637 (3C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.84 (s, 3H, OCH_3), 7.28-8.30 (m, 10H, Ar-H), 8.54 (s, 1H, coumarin-H_4), 9.06 (s, 1H, CH=N), 11.07 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.4, 104.0, 116.4, 119.6 (2C), 123.2 (2C), 124.4, 126.2 (2C), 128.4 (2C),

129.0, 132.1, 133.2, 134.5, 136.3, 138.5, 143.1, 153.5, 154.6, 156.3, 160.0, 168.5, 186.2; MS (EI, 70 eV) *m/z* (%) = 424 (M^+, 20.68), 349 (53.93), 306 (24.61), 276 (17.28), 228 (30.89), 213 (21.20), 182 (19.90), 152 (18.59), 126 (43.46). Anal. Calcd for $C_{25}H_{16}N_2O_5$ (424.41): C, 70.75; H, 3.80; N, 6.60%. Found: C, 70.71; H, 3.75; N, 6.52%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-2,4,7-trioxo-1,3,4,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-6-carbohydrazide (16).** Orange powder; yield (76%); mp 270-272 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3453-3206 (3NH), 1709-1642 (4C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.97 (s, 3H, OCH_3), 7.29-8.34 (m, 6H, Ar-H), 8.54 (s, 1H, coumarin-H_4), 9.02 (s, 1H, CH=N), 10.32, 11.18, 11.74 (s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.6, 88.4, 104.2, 119.5 (2C), 123.1, 124.3, 126.5, 128.2, 129.4, 132.0, 133.0, 143.2, 153.5 (2C), 155.0, 156.8, 160.5 (2C), 168.5; MS (EI, 70 eV) *m/z* (%) = 406 (M^+, 10.51), 375 (11.54), 276 (9.08), 241 (8.17), 182 (6.74), 126 (8.17). Anal. Calcd for $C_{20}H_{14}N_4O_6$ (406.35): C, 59.12; H, 3.47; N, 13.79%. Found: C, 59.03; H, 3.41; N, 13.71%.

***N*-((2-Methoxynaphthalen-1-yl)methylene)-4,7-dioxo-2-thioxo-1,3,4,7-tetrahydro-2*H*-pyrano[2,3-*d*]-pyrimidine-6-carbohydrazide (17).** Brown powder; yield (78%); mp > 300 ºC (EtOH-DMF); IR (KBr): ν/cm^{-1}= 3139 (3NH), 1709-1641 (3C=O), 1140 (C=S); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.97 (s, 3H, OCH_3), 7.78-8.35 (m, 6H, Ar-H), 8.56 (s, 1H, coumarin-H_4), 8.92 (s, 1H, CH=N), 10.32, 11.97, 12.53 (s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 55.6, 88.5, 104.1, 119.2 (2C), 123.2, 124.3, 126.6, 128.2, 129.5, 132.2, 133.0, 143.2, 153.5, 157.1, 159.1, 160.5, 165.0, 168.5, 170.4; MS (EI, 70 eV) *m/z* (%) = 422 (M^+, 7.31), 391 (6.81), 348 (7.31), 276 (10.84), 227 (23.71), 184 (7.19), 126 (9.58). Anal. Calcd for $C_{20}H_{14}N_4O_5S$ (422.41): C, 56.87; H, 3.34; N, 13.26%. Found: C, 56.83; H, 3.28; N, 13.21%.

ANTITUMOR EVALUATION

The stock samples were diluted with RPMI-1640 medium to desired concentrations ranging from 1 to 50 µg/mL. The final concentration of dimethyl sulfoxide (DMSO) in each sample did not exceed 1% v/v. The cytotoxic activity of the compounds was tested against human hepatocellular carcinoma (liver) cell line (HepG-2), human colorectal carcinoma (colon) cell line (HCT-116), human mammary gland (breast) cell line (MCF-7), and human epidermoid carcinoma (larynx) cell line (Hep-2). The % viability of cell was examined visually. 5-Fluorouracil was used as a standard anticancer drug for comparison.

Briefly, cell were batch cultured for 10 d, then seeded in 96- well plates of 10 x 103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Shedon.TC2323.Cornelius, OR, USA). The medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations ranging from 1 to 50 µg/mL. Cells were suspended in RPMI-1640 medium, 1% antibiotic-antimycotic mixture (104

μg/mL potassium penicillin, 104 μg/mL streptomycin sulfate and 25 μg/mL Amphotericin B) and 1% L-glutamin in 96-well flat bottom microplates at 37 ºC under 5% CO_2. After 96 h of incubation, the medium was again aspirated, trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, and examined. The cytotoxic activity was identified as confluent, relatively unaltered mono-layers of stained cells treated with compounds.

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