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Synthesis and biological evaluation of novel isoxazoles and triazoles linked 6-hydroxycoumarin as potent cytotoxic agents

Shakeel-u-Rehman [a], Masood-ur-Rahman [b], Vijay K. Tripathi [c], Jasvinder Singh [d], Tabassum Ara [b], Surrinder Koul [c,*], Saleem Farooq [c,*], Anupurna Kaul [d]

[a] Bioorganic Chemistry Division, Indian Institute of Integrative Medicine (CSIR), Srinagar 190005, India
[b] Department of Chemistry, National Institute of Technology, Srinagar 190006, India
[c] Bioorganic Chemistry Division, Indian Institute of Integrative Medicine (CSIR), Jammu 180001, India
[d] Cancer Pharmacology Division, Indian Institute of Integrative Medicine (CSIR), Jammu 180001, India

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ABSTRACT

A new series of diverse isoxazoles and triazoles linked 6-hydroxycoumarin (**1**) were synthesized using click chemistry approach. All the derivatives were subjected to 3-(4,5-dimethylthiazol-yl)-diphenyl tetrazoliumbromide (MTT) cytotoxicity screening against a panel of five different human cancer cell lines viz. prostate (PC-3), colon (HCT-116 and Colo-205), leukemia (HL-60) and lung (A-549) to check their cytotoxic potential. Interestingly, among the tested molecules, some of the analogs displayed better cytotoxic activity than the parent 6-hydroxycoumarin (**1**). Of the synthesized isoxazoles, compounds **10** and **13** showed the best activity with IC_{50} of 8.2 and 13.6 μM against PC-3 cancer cell line, while as, among the triazoles, compounds **23** and **25** were the most active with the IC_{50} of 10.2 and 12.6 μM against A-549 cancer cell line. The other derivatives showed almost comparable activity with that of the parent molecule. The present study resulted in identification of ortho substituted isoxazole and triazole derivatives of 6-hydroxycoumarin as effective cytotoxic agents against prostate (PC-3) and lung (A-549) cancer cell lines, respectively.

Coumarins are plant-derived natural products known for their diverse pharmacological properties such as anti-inflammatory, anticoagulant, antimicrobial, antiviral, anticancer, antihypertensive, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant and neuroprotective properties.[1] In addition, these compounds are also used as additives to cosmetics and food articles.[2] Natural coumarins and their derivatives are of great interest due to their widespread pharmacological properties, and this has attracted many medicinal chemists for further derivatization and screening them as novel therapeutic agents. The coumarin ring system, present in a large number of natural products (such as the anticoagulant, Warfarin), having interesting pharmacological properties[3,4] has intrigued chemists for decades to explore the natural coumarins or their synthetic derivatives for their applicability as drugs. Many intersecting molecules having coumarin based ring systems have been synthesized utilizing novel synthetic techniques. Some new derivatives bearing coumarin ring including the furanocoumarins (imperatorin), pyranocoumarins (seselin) and coumarin sulfamates (coumates) have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy.[5,6] Among the diverse biological activities of coumarins, the notable one being their effect against breast cancer and sulfatase and aromatase inhibitory activity.[7,8]

Among the coumarins, imperatorin has been found to show anticancer effects.[9] Osthole has also been found to be effective in inhibiting the migration and invasion of breast cancer cells by wound healing and transwell assays.[10] Esculetin has exhibited antitumor activities[11] and has been found to rescues cultured primary neurons from *N*-methyl-D-aspartate toxicity.[12] Chartreusin has been found to exhibit antitumor properties against murine L1210, P388 leukemias, and B16 melanoma.[13] 3″-Demethylchartreusin is a novel antitumor antibiotic produced by *Streptomyces chartreusis*.[14] In addition to this, grandivittin, agasyllin, aegelinol benzoate have been found to be cytotoxic against the A549 lung cancer cell line.[15]

Triazoles are of great importance in medicinal chemistry and are reported to possess diverse pharmacological activities such as anticancer,[16–21] immunosuppressant,[22,23] antiviral, antimicrobial, anticonvulsant, anti-inflammatory activities etc.[24] They have also been reported to be inhibitors of glycogen synthase kinase-3,[25]

* Corresponding authors. Tel.: +91 191 2569021; fax: +91 191 25693333.
E-mail addresses: skoul@iiim.ac.in (S. Koul), farooqprince100@yahoo.in (S. Farooq).

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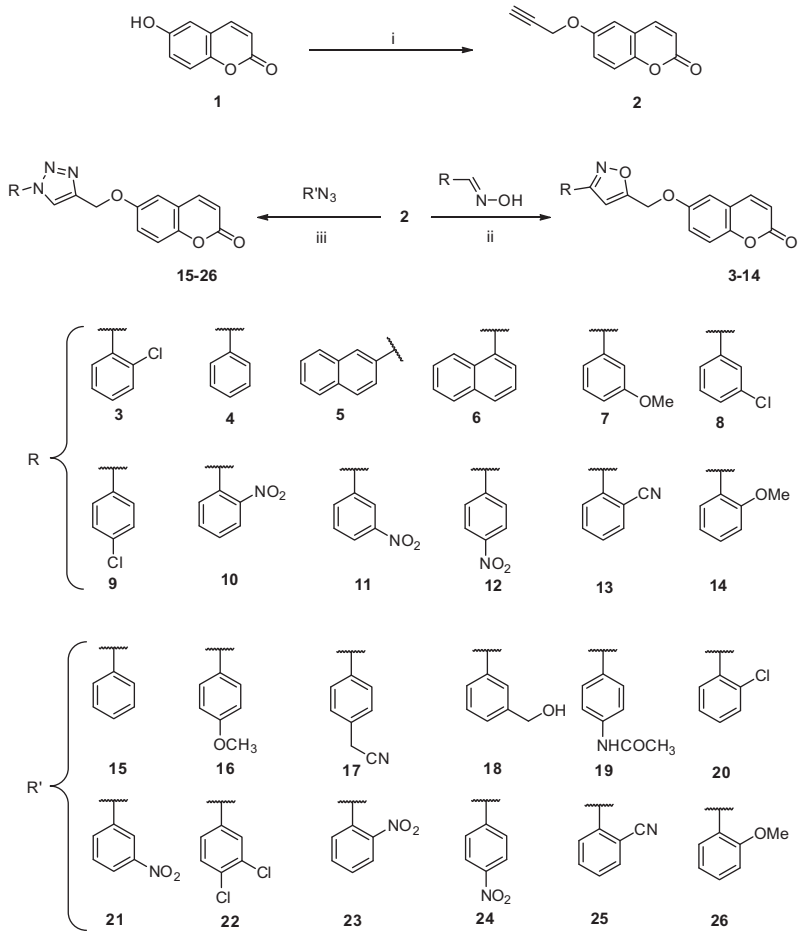
antagonists of GABA receptors[26,27] and agonists of muscarine receptors.[28]

Isoxazole and some naturally occurring isoxazole derivatives have attracted considerable attention from organic and medicinal chemists due to their considerable biological activities. For example, ibotenic acid derived from the mushroom *Amanita muscaria* is a potent neurotoxin.[29–31] Muscimol, produced naturally in the *Amanita muscaria*,[31] is an agonist of γ-aminobutyric acid A receptor ($GABA_AR$) which plays a role in regulating neuronal excitability in the central nervous system.[32] Isoxazole forms the basis for several drugs such as leflunomide (antirheumatic), valdecoxib (COX-2 inhibitor) and zonisamide (anti-convulsant). Besides, various isoxazole derivatives are reported to possess potent anticancer activity against Colo-205 and A-549 cancer cell lines.[18]

Based on the above cited findings and inspiration from the potential anticancer activity of isoxazoles and triazoles,[16–21] we directed this work towards the synthesis of a diverse series of novel isoxazolyl (**3**–**14**) and triazolyl (**15**–**26**) derivatives of biological interest using 6-hydroxycoumarin (**1**) as a key starting material.[33] The targeted compounds **3**–**14** and **15**–**26** were synthesized as depicted in Scheme 1. Click chemistry inspired approach involving union of terminal alkynes with organic oximes and azides has been taken up for the synthesis of respective regioselective[35–37] novel isoxazolyl and triazolyl derivatives of 6-hydroxycoumarin in excellent yields (85–95%). These click reactions exhibit remarkably broad scope and exquisite selectivity and have contrasting applications in chemistry, biology and materials science. The incorporation of heterocyclic moieties either as substituent group or as a fused component into parent coumarin nucleus alters its properties and converts it into a more useful product.[38]

6-Hydoxycoumarin was subjected to alkylation at hydroxyl position using propargyl bromide in presence of K_2CO_3 (base) in acetone to form 6-(prop-2-ynyloxy)-2*H*-chromen-2-one **2** (Scheme 1). The proposed structure (**2**) could easily be confirmed by the appearance of two proton singlets at δ 2.56 and 4.74 (integrating for one and two protons, respectively) assigned to terminal alkyne proton and two methylenic protons, respectively. Elemental analysis and HR-ESIMS (*m*/*z* 201.0544) data along with the ^{13}C NMR data confirmed its structure. Using 6-(prop-2-ynyloxy)-2*H*-chromen-2-one (**2**) as a key template, it was subsequently subjected to 3+2 cycloaddition with various organic oximes in presence of N-chlorosuccinimide in tetrahydrofuran to yield corresponding isoxazole derivatives (**3**–**14**) as shown in Scheme 1. The products were characterised on the basis of spectroscopic data (^1H NMR, ^{13}C NMR and HR-ESIMS) and elemental analyses. Similarly, compound **2** was allowed to undergo 1,3-dipolar cycloaddition reaction typically called Huisgen cycloaddition with various aromatic azides under sharpless click chemistry conditions ($CuSO_4{\cdot}5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O (1:1)) to afford regioselectively 1,4-disubstituted-1,2,3-triazoles (**15**–**26**) in good to excellent yields (Scheme 1). Under click conditions a series of such analogs was synthesized to look for structure–activity relationship studies. The structures of all the synthesised triazolyl derivatives were characterised by analytical and spectral data analysis. Formation of triazoles could easily be confirmed by a



Scheme 1. Reagents and conditions: (i) propargyl bromide, K_2CO_3, acetone, rt, 1 h; (ii) K_2CO_3, N-chlorosuccinimide, THF, rt, 1–2 h; (iii) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-butanol/H_2O (1:1), rt, 30 min −1 h.